REPRODUCTIVE TRACT INFECTION IN WOMEN ATTENDING OBSTETRICS AND GYNAECOLOGY DEPARTMENT OF A TERTIARY CARE HOSPITAL IN BIHAR

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ABSTRACT

BACKGROUND

Reproductive tract infection (RTI) is a common problem among women and represents a threat to their health. RTI including sexually transmitted disease (STD) and HIV/ AIDS are increasingly being recognised as a serious public health problem. The most common long-term sequelae are pelvic inflammatory disease (PID), cervical cancer, infertility, spontaneous abortion and ectopic pregnancy, which may lead to maternal death. The common infective agents are *Gardnerella vaginalis, Trichomonas vaginalis, Candida* species and *Neisseria gonorrhoeae*. Agents such as toxoplasma, rubella, CMV and HSV are important causes of infections during pregnancy. Most of the TORCH infections cause mild maternal morbidity, but have serious foetal consequences.

MATERIALS AND METHODS

Study Design**-** This prospective observational study was conducted in the Department of Microbiology, in a tertiary care medical college hospital in eastern Bihar. A total of 200 high vaginal swabs were collected in duplicate from an equal number of patients attending Obstetrics and Gynaecology Department with symptoms suggestive of RTI. In the laboratory one swab was used for culture and identification of the organism, the other was used for direct microscopic examination (Gram stain and wet mount) and other tests like amine test and measurement of pH.

RESULTS

Maximum patients were seen in the age group of 21 - 30 years, 43.5% (87/200). Least number of cases were seen in the age group of < 20 years 2.5% (5/200). Out of the 200 samples processed in the laboratory, 88 were found to show no growth. The most common isolate was *Candida* species, 25.5% (51/200). *Neisseria gonorrhoeae* 0.5% (1/200) was the least common organism isolated. Moreover, the overall rate of isolation of organisms was higher in patients with bad obstetric history.

CONCLUSION

Vulvovaginal candidiasis was the most common RTI followed by trichomoniasis and bacterial vaginosis. A single case of gonorrhoea was detected. Interestingly, the incidence of RTI was higher in patients with bad obstetric history.

KEYWORDS

Reproductive Tract Infection, Pelvic Inflammatory Disease, Bad Obstetric History.

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BACKGROUND

Reproductive tract infection (RTI) is a common problem among women and represents a threat to their health. Various socio-economic factors are responsible for the development of this disease entity, especially in a country like ours. Infection in the vulva, vagina or cervix represents lower reproductive tract infection and infection in uterus, fallopian tubes and ovaries are considered as upper reproductive tract infection.[1]

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RTI including sexually transmitted disease (STD) and

RTI including sexually transmitted disease (STD) and HIV/ AIDS are increasingly being recognised as a serious public health problem. The poor health of Indian women is a concern at both national and individual level. Women are not only more susceptible than men to these infections, but also are more prone to develop complications because infection in women is difficult to diagnose and therefore more likely to go untreated.[2]

RTIs often cause discomfort and lost economic productivity. The most common long-term sequelae are pelvic inflammatory disease (PID), cervical cancer, infertility, spontaneous abortion and ectopic pregnancy which may lead to maternal death. The presence of a sexually transmitted infection increases the risk of acquiring and transmitting HIV infection by three to five times and bacterial vaginosis may be a cofactor for HIV transmission, especially among younger women.[3]

The common infective agents are *Gardnerella vaginalis, Trichomonas vaginalis, Candida* species and *Neisseria*

gonorrhoeae. Agents such as Toxoplasma, rubella, CMV and HSV are important causes of infections during pregnancy.

Most of the TORCH infections cause mild maternal morbidity, but have serious foetal consequences.[4]

It is well realised that at least 12% - 15% of all recognised conceptions end in miscarriage and pre-clinical pregnancy loss rate is still higher (22% - 30%). At present, there is no implicating evidence that bacterial and fungal infections can cause recurrent abortions.[5] Studies have shown that these infections predispose the patients to acquisition of viral infections like HSV-2 and HIV, which in turn can lead to premature birth and spontaneous abortions.[6]

Reproductive tract infection is the commonest cause of morbidity in the reproductive age group, but the actual magnitude of the problem is grossly underestimated due to serious problems like social stigma. It is therefore imperative that proper initiation must be taken by health care providers to facilitate the diagnosis and treatment of such cases.

The present study was taken up with a view to determining the spectrum of microorganisms responsible for causing reproductive tract infection in our hospital setting and to find a possible correlation between their isolation pattern and obstetric history of the patient.

MATERIALS AND METHODS

This prospective observational study was carried out in a tertiary care medical college hospital in eastern Bihar. Institutional Ethical Committee clearance was taken before conducting the study. A total of 200 high vaginal swabs were collected in duplicate from an equal number of patients attending Obstetrics and Gynaecology Department with symptoms suggestive of RTI. A brief clinical history regarding occupation, personal hygiene etc. was noted. Obstetric history and history of any antibiotic uptake either topically or systemically was also noted down. Swabs collected were immediately sent to the laboratory for further processing.

In the laboratory one swab was used for culture and identification of the organism, the other was used for direct microscopic examination (Gram stain and wet mount) and other tests like Whiff test (amine test) and measurement of pH.[7]

Gram stained smears were further scored as per scoring system of Nugent et al. A score of ≥ 7 was indicative of bacterial vaginosis, score of 4 - 6 was taken as intermediate and score of 0 - 3 was considered as normal.[8]

Identification of the organisms isolated was done by study of colony morphology, Gram's staining and motility followed by a battery of biochemical tests.[7,9]

Neisseria gonorrhoeae was identified by colony morphology on chocolate agar, Gram's stain morphology, catalase, oxidase and rapid carbohydrate utilisation tests.[7]

Gardnerella vaginalis was identified on the basis of presence of β-haemolytic colonies on human blood Tween 80 bi-layer medium, presence of Gram variable bacilli on Gram staining, starch and raffinose fermentation and hippurate hydrolysis tests.[8]

Candida species was identified by germ tube test, chlamydospore formation on cornmeal agar, sugar fermentation and sugar assimilation test.[7]

Statistical analysis of results was done using the chi-square test. P-value ≤ 0.05 was considered to be significant and p-value ≤ 0.001 was considered to be highly significant. All statistical analysis was carried out using online statistical software at-

http://www.physics.csbju.edu/stats/contingency_NROW_NCOLUMN_form.html; accessed on 14.02.2018. All media and reagents were procured from HiMedia Laboratories, Mumbai.

RESULTS

A total of 200 females were inducted into the study, out of which maximum number of patients were seen in the age group of 21 - 30 years, 43.5% (87/200) followed by age group 31 - 40 years, 39.0% (78/200). Least number of cases were seen in the age group of < 20 years 2.5% (5/200) [Table 1].

Out of 200 women, 84.5% (169/200) were multiparous, only 15.5% (31/200) were nulliparous. Among the multiparous women 157 had a good obstetric history (GOH), while 12 had a bad obstetric history (BOH) [Table 2].

On microscopic examination of vaginal swabs 3% (6/200) patients showed presence of clue cells, 10% (20/200) showed presence of *Trichomonas sp.* and yeast-like cells were seen in 31% (62/200) of samples [Table 3].

Overall, RTI was seen in 48.5% (97/200) of patients in the study group, whereas 44.0% (88/200) showed no growth. The most common isolate was Candida species 25.5% (51/200) followed by non-albicans candida species 5.5% (11/200) and *Gardnerella vaginalis* 3% (6/200). *Neisseria gonorrhoeae* 0.5% (1/200) was the least common organism isolated. *Trichomonas vaginalis* could be detected by wet mount examination in 10% (20/200) of patients. The overall rate of isolation of organisms was higher in patients with BOH. The differences in rate of isolation of various organisms in patients with GOH, BOH and nullipara was found to be highly significant (p= 0.000) [Table 4].

As per Nugent criteria, 7% (14/200) of patients were found to have bacterial vaginosis. Out of these 14 patients, both Whiff test and vaginal pH ≥ 6 was found to be positive in 85.7% (12/14) and *Gardnerella vaginalis* could be grown in only 42.9% (6/14) of cases [Table 5].

Age	No. of Married Patients (%)	No. of Unmarried Patients (%)	Total
≤ 20	2 (1.0)	3 (100.0)	5 (2.5)
21-30	87 (44.2)	0	87 (43.5)
31-40	78 (39.6)	0	78 (39.0)
41-50	30 (15.2)	0	30 (15.0)
Total	**197**	**3**	**200**

Table 1. Age-Wise Distribution of Married and Unmarried Patients

Parity	No. of Patients	Percentage
Multiparous with good obstetric history	157	78.5
Multiparous with bad obstetric history	12	6.0
Nulliparous	31*	15.5
Total	**200**	**100.0**

Table 2. Obstetric History of Patients with RTI

*All married and unmarried women who never conceived were included in nullipara.

Diagnostic Procedure	Clue Cells (%)	Trophozoites of Trichomonas (%)	Yeast-Like Organisms (%)	No. Relevant Findings (%)
Wet-mount (n= 200)	6 (3.0)	20 (10.0)	62 (31.0)	112 (56.0)
Gram stain (n= 200)	10 (5.0)	NA	62 (31.0)	128 (64.0)

Table 3. Microscopic Findings in Patients with RTI

Organisms Isolated/Detected	Multiparous with Good Obstetric History (%)*	Multiparous with Bad Obstetric History (%)*	Nulliparous (%)*	Total No. (%)
Candida albicans	44 (28.0)	4 (33.3)	3 (9.7)	51 (25.5)
Non-albicans Candida species	8 (5.1)	2 (16.7)	1 (3.2)	11 (5.5)
Gardnerella vaginalis‖	5 (3.2)	1 (8.3)	0 (0.0)	6 (3.0)
*Trichomonas vaginalis***	17 (10.8)	3 (25.0)	0 (0.0)	20 (10.0)
Neisseria gonorrhoea	0 (0.0)	1 (8.3)	0 (0.0)	1 (8.3)
Growth of normal flora	17 (10.8)	1 (8.3)	5 (16.1)	23 (11.5)
Sterile	66 (42.0)	0 (0.0)	22 (71.0)	88 (44.0)
Total*	**157**	**12**	**31**	**200**

Table 4. Isolation Pattern of Different Organisms in Patients with RTI

- ‖isolated in only 6 of the 14 cases of bacterial vaginosis **detected by microscopy only.
- *p value= 0.000.

Patients with Bacterial Vaginosis (n= 14)	Whiff Test	Vaginal pH			Nugent Scoring			Growth of Gardnerella Vaginalis
		pH ≤ 4	pH 4-6	pH ≥ 6	0-3	4-6	≥ 7	
No. of Positive Cases (%)	12 (85.7)	00	02 (14.3)	12 (85.7)	00	00	14 (100.0)	06 (42.9)
No. of Negative Cases	02 (14.3)	14 (100.0)	12 (85.7)	02 (14.3)	14 (100.0)	14 (100.0)	00	08 (57.1)

Table 5. Positivity of Various Tests in Patients with Bacterial Vaginosis

DISCUSSION

Majority of the patients (43.5%) were in the age group of 21-30 years, followed by the age group of 31 - 40 years (39%). Least number of patients (2.5%) belonged to the age group of ≤ 20 years. This is probably due to the fact that RTIs are more common in the sexually active reproductive age group. Most of the patients, 197/200 were married. Other authors have also reported a higher rate of RTI in married patients as compared to unmarried patients.[10]

Majority of the patients were multiparous 84.5% (169/200), only 15.5% (31/200) were nulliparous which included three unmarried females. Of the multiparous women, 12/200 had BOH. Another author also reported that incidence of RTI increases with parity. RTI was least common in nulliparous (6%) and highest (76%) in multiparous women.[11]

Candida species were the most common isolate with an isolation rate of 25.5% (51/200) for *Candida albicans* and 5.5% (11/200) for non-albicans candida species. *Neisseria gonorrhoeae* of 0.5% (1/200) was the least common isolate. 11.5% (23/200) samples showed mixed bacterial growth, while 44% (88/200) samples were sterile. The high percentage samples showing no growth in culture were probably due to use of self-medication or use of ointments or lotions by the patient. Bacterial vaginosis was seen in 7% (14/200).

Patnaik and Sahu (2008) has reported Candidiasis in 33.9% of cases, Bacterial vaginosis in 14.3%, *Trichomoniasis* in 12.5% and *N. gonorrhoeae* in 1.8%.[12] Similarly, Madhivanan and Bartman (2009) have reported the prevalence of *T. vaginalis* as 8.5% as compared to 10% in our present study.[13] In another study the incidence of bacterial vaginosis was reported as 20%, Candidiasis as 12.5%, *N. gonorrhoeae* as 1.2% and *T. vaginalis* as 4.1%. These findings are quite different from those of the present study.[14]

The rate of isolation of various organisms from patients with BOH was higher as compared to those with GOH. *Candida albicans* was seen in 33.33% of patients with BOH as compared to 28.03% in GOH, non-albicans *Candida* species in 16.67% of patients with BOH versus 5.09% in GOH. *Gardnerella vaginalis* 8.33% in BOH versus 3.18% in GOH. *Trichomonas vaginalis* was seen in 25% of patients with BOH as compared to 10.83% of patients with GOH.

The differences in isolation rates of organisms in the three groups of patients with RTI viz. multiparous with GOH, multiparous with BOH and nulliparous was found to be highly significant (p value < 0.001). The higher rate of isolation from patients with BOH is probably due to the fact that although these organisms alone are not very important causes of PID, their presence predisposes carriers to acquire other STIs such as HIV and HSV2 virus that have been implicated in causation of endometritis (PID), resultant BOH and infertility.

CONCLUSION

The institution where this study was conducted is located in a small town in eastern Bihar and mainly caters to the rural population of the adjoining areas. This area is part of the Kosi belt, a region known for its poor developmental activities. Consequently, the general population residing here, especially the females are illiterate with poor personal hygiene and occurrence of RTI is a common ailment. In the present study, RTI was seen in 48.5% (97/200) of patients. Vulvovaginal candidiasis was the most common RTI, followed by *trichomoniasis* and bacterial vaginosis. A single case of gonorrhoea was detected.

Studies have shown that common bacterial and fungal agents responsible for RTI do not directly lead to premature

births or recurrent abortions, but their presence predisposes the patients to acquisition of sexually transmitted infections like HSV-2 and HIV, both of which can lead to complications during pregnancy. In our study, the incidence of RTI was higher in the patients with BOH which corroborates the above theory.

Hence, efforts should be made for early diagnosis and treatment of RTIs, as it not only leads to morbidity and distress in patients but it may result in complications during pregnancy, PID and sometimes infertility.

Awareness and sensitisation towards the problem, so that women take proper and complete course of medications rather than reverting to traditional methods of disease control is the need of the hour. New methodologies and interventions should be set up both at service provider level as well as beneficiary end to bring down prevalence rates of RTI.

REFERENCES

[1] Gaash B, Kausar R, Bhan R, et al. Reproductive tract infection in Kargil: a community based study. Indian Journal of Practicing Doctors 2004;1(2):342-4.

[2] Srivastava A, Nandan D, Mehrotra AK, et al. A comparative study of perception about reproductive tract infections among married women in rural, urban and urban slum areas. Indian Journal of Community Medicine 2004;29(2):274-8.

[3] Prasad JH, Abraham S, Kurz KM, et al. Reproductive tract infections among young married women in Tamil Nadu, India. International Family Planning Perspectives 2005;31(2):73-82.

[4] Aljumaili ZKM, Alsamarai AM. Risk factors for bad obstetric history in Kirkuk women, Iraq. Int J Infect Microbiol 2013;2(3):70-7.

[5] Devi R, Srinivas N, Rajangam S. Bad obstetric history and infectious causes. Int J Hum Genet 2002;2(4):269-71.

[6] Agwu AL, Jang SS, Korthuis PT, et al. Pregnancy Incidence and outcomes in vertically and behaviourally HIV-infected youth. JAMA: The Journal of American Medical Association 2011;305(5):468-70.

[7] Winn W, Allen S, Janada W, et al. In: Koneman's Color Atlas and Textbook of diagnostic microbiology. 6th edn. Philadelphia: Lippincott Williams & Wilkins 2006:87-91, 576-95, 1216-20.

[8] Howard AJ, Catherine AI. Haemophilus, Gardnerella and other bacilli. In: Collee JG, Fraser AG, Marmion BP, et al. eds. Mackie and McCartney Practical medical microbiology. 14th edn. New Delhi: Churchill Livingstone 2008: p. 456-8.

[9] Collee JG, Miles RS, Watt B. Tests for Identification of Bacteria. In: Collee JG, Fraser AG, Marmion BP, et al. eds. Mackie and McCartney Practical medical microbiology. 14th edn. New Delhi: Churchill Livingstone 2008: p. 131-49.

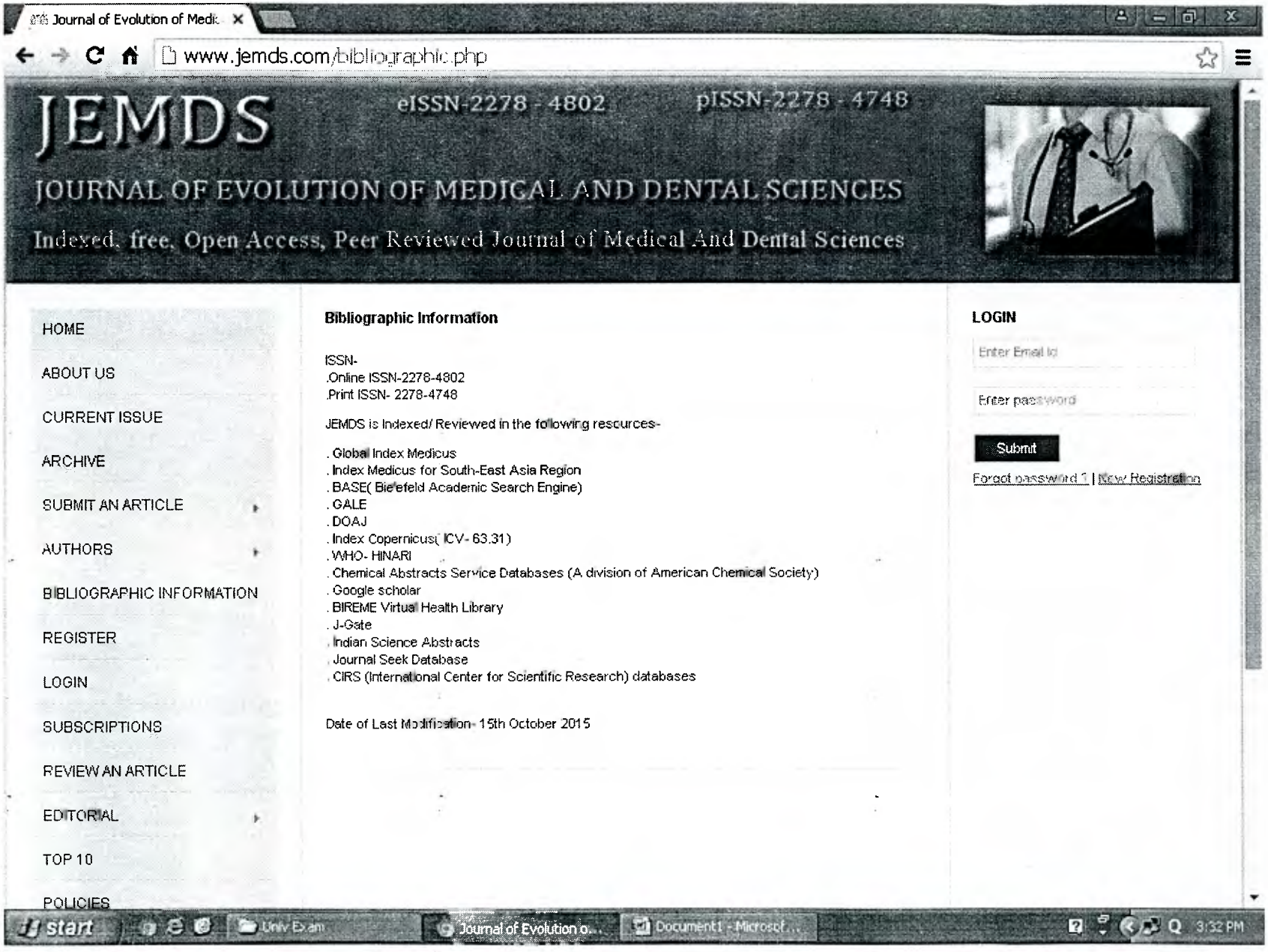
[10] Ram R, Bhattacharya K, Goswami DN, et al. Syndromic approach for determination of reproductive tract infections among adolescent girls. Journal of the Indian Medical Association 2006;104(4):178, 180-1.

[11] Rathore M, Vyas L, Bhardwaj AK. Prevalence of reproductive tract infections among women and socio-cultural factors associated with it. Journal of the Indian Medical Association 2007;105(2):71-2, 74, 78.

[12] Patnaik L, Sahu T, Sahani NC. Syndromic diagnosis of RTI/STI among women of reproductive age group. Indian Journal of Community Medicine 2008;4(1):248-54.

[13] Madhivanan P, Bartman MT, Pasutti L, et al. Prevalence of Trichomonas vaginalis infection among young reproductive age women in India: implications for treatment and prevention. Sex health 2009;6(4): 339-44.

[14] Oliviera FA, Pfleger V, Lang K, et al. Sexually transmitted infections, bacterial vaginosis and candidiasis in women of reproductive age in rural Northeast Brazil: a population- based study. Mem Inst Oswaldo Cruz 2007;102(6):751-6.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ORIGINAL ARTICLE

EXTENDED SPECTRUM B-LACTAMASE PRODUCTION AMONGST GRAM NEGATIVE BACILLI ISOLATED FROM PATIENTS ATTENDING A TERTIARY CARE HOSPITAL IN EASTERN BIHAR.

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ABSTRACT: BACKGROUND: Extended Spectrum β-lactamases (ESBL) are enzymes that have the ability to hydrolyze β-lactam antibiotics containing oxyimino group and are inhibited by β-lactamase inhibitors. These enzymes are responsible for resistance to penicillins, monobactams, third generation and in some instances fourth generation cephalosporins. ESBLs are encoded by transferable conjugative plasmids that often code for resistance to other antibiotics as well. **OBJECTIVE:** This prospective study aimed to determine ESBL production in Gram negative bacteria in patients attending a tertiary care hospital in eastern Bihar. **METHOD:** A total of 556 samples from patients attending inpatient and outpatient departments from May 2009 to April 2010 were included in the study. Samples were processed as per standard protocol and antibiotic susceptibility testing was done by modified Kirby–Bauer method. Isolates showing resistance to any third generation cephalosporin were subjected to Double Disc Synergy Test (DDST), Phenotypic Confirmatory Disc Diffusion Test (PCDDT) and MIC reduction test for ESBL production. **RESULTS:** 42.62% of Gram negative bacilli were ESBL producers. 56.3% of Klebsiella pneumoniae strains were found to be ESBL producers whereas only 40.5% of *Escherichia coli* produced ESBL. PCDDT and MIC reduction test showed 100% correlation whereas the DDST failed to detect 13.5% of ESBL producers. All ESBL producers were sensitive to Imipenem and Cefoperazone/ Sulbactam. **CONCLUSION:** The present study gives us an indication regarding the occurrence of ESBL producing Gram negative bacilli in Eastern Bihar. The number of ESBL producers in this region is alarmingly large. It is therefore recommended that PCDDT be incorporated in all laboratories as a part of routine antibiotic susceptibility testing procedures as it is simple, reliable and reproducible test for detection of ESBLs.
KEYWORDS: Extended Spectrum β-lactamases, ESBL, DDST, PCDDT.

INTRODUCTION: Extended Spectrum β-lactamases (ESBL) are enzymes that have the ability to hydrolyze β-lactam antibiotics containing an oxyimino group (third generation cephalosporin and Aztreonam) and are inhibited by β-lactamase inhibitors like Clavulanic acid, Sulbactam and tazobactam[1]. Production of ESBLs lead to resistance to penicillins, monobactams, third generation cephalosporins like Cefotaxime, Ceftriaxone, Ceftazidime and in some instances fourth generation cephalosporins also[2].

Emergence of resistance to β-lactam antibiotics began even before the first β-lactam antibiotic penicillin was developed[3]. The first plasmid mediated β-lactamase, TEM-1 was reported in 1965 from an Escherichia coli isolate belonging to a patient in Athens, Greece named Temoniera [hence designated TEM][4]. Over the years, many new β-lactam antibiotics have been developed; however, with each new class of antibiotic, a new β-lactamase emerged that caused resistance to that class of drug. Presumably, the selective pressure imposed by the use and overuse of new antibiotics results in the emergence of new variants of β-lactamases[5]. The first report of plasmid-encoded β-lactamase capable of hydrolyzing the extended spectrum cephalosporins was published in 1983 from Germany[6]. Hence these new β-lactamases were coined as extended spectrum β-lactamases[7]. Over the past few decades, a number of new β-lactamases in clinical isolates of members of the family Enterobacteriaceae have emerged. The total number of ESBLs characterized exceeds 200 today[8]. ESBLs are encoded by transferable conjugative plasmids which often code for resistance to other antibiotics as well[7]. Being plasmid mediated, they are easily transmitted among the members of Enterobacteriaceae family, thus facilitating the dissemination of resistance not only to β- lactams but also to other commonly used antibiotics such as quinolones and aminoglycosides[9]. Major risk factors for colonization or infection with ESBL producing organisms are prolonged exposure to antibiotics, prolonged ICU stay, nursing home residency, severe illness, catheterization, instrumental intervention and residence in an institution with high rate of use of third generation cephalosporins.[5]

This prospective study aimed at determining ESBL production in Gram negative bacilli in a tertiary care hospital in eastern Bihar. Detection of ESBL production is important because it ultimately determines the clinical outcome in patients infected with such strains. Studies on ESBL producing Gram negative bacilli have been reported from all parts of India however, to the best of our knowledge; no such studies till date have been reported from Bihar and Jharkhand.

MATERIAL AND METHODS: Clearance from Institutional Ethics Committee was obtained prior to carrying out this study. A total of five hundred and fifty six (556) samples viz. urine, pus, stool and pleural fluid from patients attending different inpatient and outpatient departments were included in the study. A brief clinical history of the patients regarding antibiotic intake, instrumentation and duration of hospital stay was taken. Specimens collected were inoculated on 5% sheep blood agar and MacConkey's agar. They were identified by standard biochemical tests.[10] Antibiotic Susceptibility Testing (AST) was done on Mueller–Hinton agar (MHA) plates by modified Kirby–Bauer disc diffusion technique using commercially available antibiotic discs (HiMedia, Mumbai). When the zone of inhibition of an isolate for any one or more of the third generation cephalosporin (Cefotaxime, Ceftriaxone, Ceftazidime & Cefoperazone) was less than or equal to the zone diameter recommended by CLSI (Clinical and Laboratory Standards Institute), the isolate was further tested by the PCDDT, MIC reduction test and DDST for ESBL production.[11]

DDST: Bacterial suspension to be tested was prepared in Mueller–Hinton Broth (MHB). After matching the turbidity to 0.5 McFarland's standard, the organism was inoculated on MHA as per guidelines for disc diffusion technique. Amoxycillin/Clavulanic acid disc was placed in the centre of the plate. Four antibiotic discs i.e. Cefotaxime, Ceftazidime, Ceftriaxone and Cefoperazone were placed at a distance of 20 mm (centre to centre) from Amoxycillin/Clavulanic acid disc and at 90° from each other. The plates were examined after

overnight incubation. Enhancement of inhibition zone for any of the four antibiotic discs towards Amoxycillin/ Clavulanic acid disc indicated the production of ESBL by the strain.

PCDDT: MHA plates were inoculated in the same manner as DDST. Four antibiotic discs viz. Cefotaxime, Cefotaxime/ Clavulanic acid, Ceftazidime and Ceftazidime/ Clavulanic acid were placed at a distance of 30 mm (centre to centre) from each other. Plates were examined after overnight incubation at 37°C. ESBL production was confirmed when there was an increase in the zone diameter by 5 mm or more when Clavulanic acid was added to the respective antibiotic. **[Fig. 1]**

MIC reduction test: Bacterial suspension to be tested was prepared in MHB and matched to 0.5 McFarland's standard. As a final concentration of 5 X 10^5 CFU/ml was required, bacterial suspension equivalent to 0.5 McFarland's standard was further diluted 1:100 in MHB.[12]

- Antibiotics in powder form were obtained from Sigma-Aldrich Chemicals Pvt. Ltd, Bangalore. Dilutions were prepared in the following range:
  - Ceftazidime : 0.25 – 256 µg/ml
  - Ceftazidime/Clavulanic acid : 0.25/4 – 256/4 µg/ml
  - Cefotaxime : 0.25 – 256 µg/ml
  - Cefotaxime/Clavulanic acid : 0.25/4 – 256/4 µg/ml

Antibiotic ranges were prepared one step higher than the final dilution range required to compensate for the addition of equal volume of inoculum. 100 µl of each antibiotic dilution was dispensed in their respective labelled wells. 100 µl of diluted bacterial suspension was added to each well. 200 µl of uninoculated and inoculated broth were also dispensed in wells in each row as sterility and growth control. After overnight incubation, the microtitre wells were examined. The lowest concentration of the antibiotic that showed no visible growth represented the MIC value of the organism. An isolate was confirmed as ESBL producer if there was ≥ 3 two folds (eight times) reduction in MIC of third generation cephalosporin on addition of Clavulanic acid as compared to third generation cephalosporin when used alone[12]. **[Fig. 2]**

RESULTS: A total of five hundred and fifty six (556) samples were received in the laboratory during the study period out of which one hundred and twenty two strains of Gram negative bacilli were isolated (21.9%). 62 strains were from indoor while 60 were from outdoor patients. Overall 52 (42.6%) of these isolates were found to be ESBL producers; 56.3% of Klebsiella pneumoniae were ESBL producers, followed by 50.0% of Klebsiella oxytoca and 40.5% of Escherichia coli. ESBL production was also seen in Pseudomonas aeruginosa (35.7%) and Proteus mirabilis (33.3%). **[Table 1]**

Most of the ESBL strains were isolated from the department of Surgery (38.5%) followed by department of Obstetrics & Gynaecology (28.9%) and department of Medicine (23.1%). Comparison of DDST and PCDDT with MIC reduction test showed that the PCDDT was superior to the DDST for detection of ESBL producers. PCDDT showed 100% correlation with MIC reduction test whereas DDST failed to detect 13.5% of ESBL producers.

ESBL producers were isolated more frequently in patients hospitalized for more than 14 days (44.8%) when compared with ≤ 7 days of hospitalization (20.7%). **[Table 2]**

All ESBL producers were resistant to Cefuroxime and Piperacillin. 90.0% of strains were resistant to Cefoperazone, 82.7% to Ceftriaxone, 76.9% to cefotaxime and 75.0% to Ceftazidime.

All the strains were sensitive to Imipenem and Cefoperazone/ Sulbactam combination. [Table 3]

DISCUSSION: The present study gives us an indication regarding the occurrence of ESBL producing Gram negative bacilli in Eastern Bihar. Unfortunately the number of ESBLs isolated in this region is alarmingly large (42.6% of all Gram negative bacilli). It was observed that majority of these ESBL producers were from indoor patients especially in those with prolonged hospital stay. Various studies have reported ESBL production to be as high as 61.7% and as low as 11.0%.[13,14,15]

Majority of ESBL producers were Klebsiella pneumoniae, this was followed by Klebsiella oxytoca and Escherichia coli. Many other studies have also reported that majority of ESBL producers were either Escherichia coli or Klebsiella pneumoniae.[13 14,15] As was seen in this study, other authors have also reported ESBL production in Klebsiella oxytoca, Pseudomonas aeruginosa, Proteus mirabilis and Citrobacter freundii. [13,14,16,17]

The PCDDT was found to give good results as far as detection of ESBL was concerned, showing 100% concordance with the MIC reduction test. Similar findings were also reported by other workers.[7]

Presence of these organisms in the hospital environment is a man-made phenomenon due to over-use and misuse of 3rd generation cephalosporins and other broad spectrum antibiotics. Proper infection control practices and formulation of a hospital antibiotic usage policy is clearly indicated. The other main issue that needs to be addressed is the incorporation of tests for detection of ESBL as a routine in all Microbiology laboratories. The PCDDT was found to be a simple, reliable and reproducible test that showed 100% conformity with the MIC reduction test which is time taking, difficult to interpret and needs a high degree of precision. The PCDDT therefore has the potential to be incorporated in all laboratories, especially smaller ones that do not have the resources to avail automated systems, as a part of routine antibiotic susceptibility testing procedures.

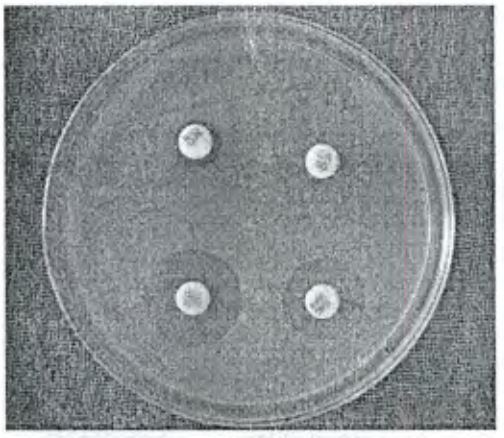

Fig.1 : Phenotypic Confirmatory Disc Diffusion Test showing enhancement of inhibition zone with corresponding disc containing clavulanic acid.

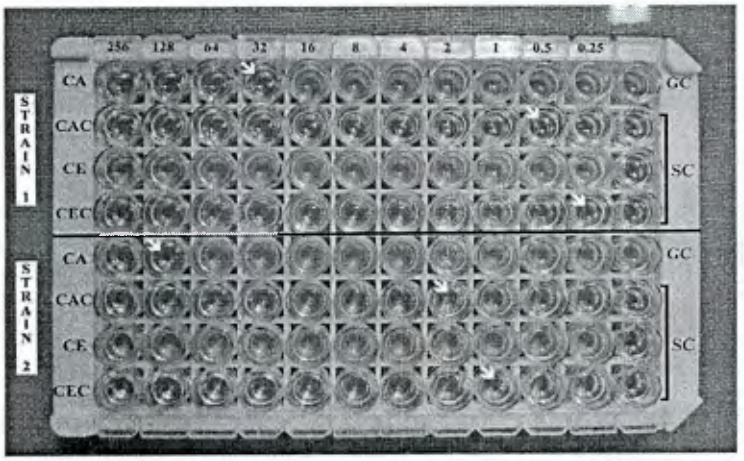

Fig.2 : Determination of Minimum Inhibitory Concentration by microdilution method.
CA: Ceftazidime CAC: Ceftazidime/clavulanic acid
CE: Cefotaxime CEC: Cefotaxime/clavulanic acid [arrows indicating MIC]
GC: Growth control SC: Sterility control

Table 1: Rate of isolation of ESBL producers among Gram negative isolates.

Isolate	ESBL (%)	Non-ESBL (%)	Total
Escherichia coli	30 (40.54)	44 (59.46)	74
Klebsiella pneumonia	09 (56.25)	07 (43.75)	16
Klebsiella oxytoca	04 (50.00)	04 (50.00)	08
Pseudomonas aeruginosa	05 (35.71)	09 (64.29)	14
Proteus mirabilis	02 (33.33)	04 (66.67)	06
Citrobacter freundii	02 (50.00)	02 (50.00)	04
Total	52 (42.62)	70 (57.38)	122

Table 2: Duration of hospital stay in relation with ESBL infection.

Duration of stay (days)	ESBL	Percentage	Non-ESBL	Percentage
≤7	06	20.69	22	66.67
8 – 14	10	34.48	06	18.18
>14	13	44.83	05	15.15
Total	29	100.00	33	100.00

Table 3: Antibiotic susceptibility pattern of ESBL and non-ESBL producing isolates.

Antibiotics	ESBL production	Resistant (%)	Intermediate (%)	Sensitive (%)	Total
Cefuroxime	ESBL	52 (100.0)	0 (0.00)	0 (0.0)	52
	Non ESBL	58 (82.9)	11 (15.71)	01 (1.4)	70
Ceftazidime	ESBL	39 (75.0)	13 (25.00)	0 (0.0)	52
	Non ESBL	18 (25.7)	10 (14.29)	42 (60.0)	70
Cefotaxime	ESBL	40 (76.9)	10 (19.23)	02 (3.8)	52
	Non ESBL	22 (31.4)	09 (12.86)	39 (55.7)	70
Ceftriaxone	ESBL	43 (82.7)	09 (17.31)	0 (0.0)	52
	Non ESBL	22 (31.4)	0 (0.00)	48 (68.6)	70
Cefoperazone	ESBL	47 (90.4)	04 (7.70)	01 (1.9)	52
	Non ESBL	20 (28.6)	10 (14.29)	40 (57.1)	70
Amikacin	ESBL	06 (11.5)	04 (7.70)	42 (80.8)	52
	Non ESBL	25 (35.7)	05 (7.15)	40 (57.1)	70
Levofloxacin	ESBL	14 (26.9)	07 (13.46)	31 (59.6)	52
	Non ESBL	27 (38.6)	11 (15.72)	32 (45.7)	70

Gentamicin	ESBL	36 (69.2)	01 (1.92)	15 (28.9)	52
	Non ESBL	41 (58.6)	09 (12.86)	20 (28.6)	70
Piperacillin	ESBL	52 (100.0)	0 (0.00)	0 (0.0)	52
	Non ESBL	24 (34.3)	21 (30.00)	25 (35.7)	70
Cefoperazone/ Sulbactam	ESBL	0 (0.0)	0 (0.00)	52 (100.0)	52
	Non ESBL	16 (22.9)	06 (8.57)	48 (68.6)	70
Piperacillin/ Tazobactam	ESBL	03 (5.8)	01 (1.92)	48 (92.3)	52
	Non ESBL	18 (25.7)	23 (32.86)	29 (41.4)	70
Imipenem	ESBL	0 (0.00)	0 (0.00)	52 (100.0)	52
	Non ESBL	0 (0.00)	0 (0.00)	100 (100.0)	70

REFERENCES:

1. Brooks GF and Carroll KC. Antimicrobial chemotherapy. In: Brooks GF, Carroll KC, Butel JS, Morse SA (eds). Jawetz, Melnick and Adelberg's Medical Microbiology, 24th ed. The McGraw-Hill Companies: New York: 2007; 162.
2. Russo AT and Johnson JR. Diseases caused by Gram negative enteric bacilli. In: Fauci AS, Kasper DL, Longo DL, Brawnwald E et al. (eds), Harrison's Principles of Internal Medicine, 17th ed, The McGraw Hill Companies: Boston: 2008; vol. I: 938.
3. Abraham EP and Chain E. An enzyme from bacteria able to destroy penicillin. Nature. 1940; 146: 837.
4. Datta N and Kontomichalou P. Penicillinase synthesis controlled by infectious R Factors in Enterobacteriaceae. Nature. 1965; 208:239–244.
5. Chaudhary U and Aggarwal R. Extended spectrum beta lactamases (ESBL) – An emerging threat to clinical therapeutics. Indian J Med Microbiol. 2004; 22(2): 75-80.
6. Knothe H, Shah P, Krcmery V et al. Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of Klebsiella pneumoniae and Serratia marcescens. Infection. 1983; 11: 315–317.
7. Agrawal Parul, Ghosh AN, Kumar Satish et al. Prevalence of extended spectrum β-lactamases among Escherichia coli and Klebsiella pneumoniae isolates in a tertiary care hospital. Indian J Pathol Microbiol. 2008; 51(1): 139-142.
8. Paterson David L and Bonomo Robert A. Extended spectrum β-lactamases: a clinical update. Clin Microbiol Rev. 2005; 18(4): 657-686.
9. Varaiya A, Dogra J. Kulkarni M et al. Extended spectrum β-lactamase producing Escherichia coli and Klebsiella pneumoniae in diabetic foot infection. Indian J Med Microbiol. 2008; 26(3): 281-282.

10. Forbes BA, Sahm DF, Weissfeld AS (eds). Overview of Bacterial Identification Methods and Strategies. In: Bailey and Scott's Diagnostic Microbiology. 12th ed. Mosby Elsevier, Missouri. 2007; 216-247.
11. CLSI. Performance standards for antimicrobial susceptibility testing: Eighteenth informational supplement, M100-S18, January. 2008; 28(1): 162-163.
12. Andrews JM. Determination of minimum inhibitory concentration. J Antimicrobiol Chemother.2001; 44: 5-16.
13. Deep and Usha Arora. Extended spectrum β-lactamases – mediated resistance among the family Enterobacteriaceae. Indian J Pathol Microbiol. 2007; 50(2): 458-461.
14. Sridhar Rao PN, Basavarajappa KG and Krishna G. Leela. Detection of extended spectrum beta-lactamase from clinical isolates in Davangere. Indian J Pathol Microbiol. 2008; 51(4): 497-499.
15. Kader Abdulrahman Abdulla and Kumar Angamuthu. Prevalence and antimicrobial susceptibility of extended spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in a general hospital. Ann Saudi Med. 2005; 25(3): 239-242.
16. Aggrawal Ritu, Chaudhary Uma and Sikka Rama. Detection of extended spectrum β-lactamase production among uropathogens. J Lab Physicians. 2009; 1(1): 7-9.
17. Bhattacharjee A, MR Sen, P Prakash et al. Increased Prevalence of Extended Spectrum Beta lactamase producers in neonatal septicemia cases at a tertiary referral hospital. Indian J Med Microbiol. 2008; 26(4): 356-360.

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ORIGINAL ARTICLE

CLINICO-MYCOLOGICAL PROFILE OF DERMATOPHYTOSIS IN PATIENTS ATTENDING A TERTIARY CARE HOSPITAL IN EASTERN BIHAR, INDIA

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ABSTRACT: BACKGROUND: Dermatophytes are closely related keratinophilic fungi that cause dermatophytosis. Dermatophytosis is caused by three genera of fungi imperfectii viz. Microsporum, Trichophyton and Epidermophyton and where the perfect state of the species has been identified to the genus Arthroderma in the class Ascomycetes. Their keratinophilic nature allows them to degrade keratin and thus invade skin, hair and nails. **AIMS:** This study aimed to establish the identity of fungal isolates from clinically suspected cases of dermatophytosis and to correlate the occurrence of dermatophytosis with clinico-epidemiological profile of patient. **MATERIALS AND METHODS** A total of 372 samples from patients attending outpatient department from March 2010 to May 2011 were included in the study. A brief clinical history was obtained from the patients and samples were collected and processed as per standard protocol. Fungal growth was identified by gross colony morphology, lactophenol cotton blue mount, and color change on dermatophyte test medium (DTM) and microslide culture. **RESULTS:** The most common dermatophyte was Trichophyton rubrum (12.1%) whereas Aspergillus niger (5.6%) was the commonest isolate among non-dermatophyte. Among clinical forms, majority of the patients had tinea corporis (55.3%). **CONCLUSIONS:** Dermatophytosis was found to be more prevalent in young adults (21-30 years). Culturing specimen on Sabouraud Dextrose Agar (SDA) with antibiotics was found to be the best method for diagnosis and this medium proved to be better than DTM. The most common dermatophyte was Trichophyton rubrum and the most common clinical form of dermatophytosis was tinea corporis. Fungal infection of the skin tends to be chronic and lead to disfigurement, which may be a source of embarrassment to the person concerned. Diagnosis of these fungal infections in the laboratory must be encouraged, as they are easy to perform and require minimum infrastructure.

KEYWORDS: Dermatophytes, Epidermophyton, Microsporum, Trichophyton.

INTRODUCTION: By broad definition, the term dermatophyte might be taken as all fungi causing disease in man and animals by invasion of the skin, but usage has tended to restrict the label of fungi capable of causing skin changes of the type known as ringworm. Thus defined the ringworm species are all moulds belonging to three genera of the fungi imperfectii viz. Microsporum, Trichophyton and Epidermophyton and where the perfect state of the species has been identified to the genus Arthroderma in the class Ascomycetes.[1]

The dermatophytes are closely related keratinophilic fungi that cause dermatophytosis (ringworm/tinea). Their keratinophilic nature allows them to degrade keratin and thus invade skin, hair and nails. The dermatophytes capable of reproducing sexually belong to the genus Arthroderma in the family Arthrodermataceae. Physiologically, dermatophytes are distinct because of their ability to tolerate high concentrations of cycloheximide and by their ability to utilize proteins as the sole source of carbon.[2]

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The prevalence of dermatophytes depends on environmental conditions, personal hygiene and individual's susceptibility from place to place. Dermatophytosis is prevalent throughout the world. Some species are endemic in certain parts of the world and have limited geographic distribution. Trichophyton soudanense, Trichophyton gourvilli and Trichophyton yaoundei are found in Central and West Africa. Microsporum ferrugineum is seen in Japan and surrounding areas. Trichophyton concentricum is confined to South Pacific, Central and South America. However, in recent time due to mass migration of people from one part of the world to another, these barriers have broken down.[3]

Dermatophytosis and other cutaneous fungal infections, tends to be a chronic, are disfiguring and is associated with social stigma. Moreover an epidemiological data regarding the prevalence and incidence of dermatophyte species actually causing these infections is not known because patients are by and large treated based on clinical diagnosis. The present study was therefore undertaken to establish the identity of fungal isolates from clinically suspected cases of dermatophytosis and to correlate the occurrence of dermatophyte infection with clinico-epidemiological profile of the patient.

MATERIALS AND METHODS: This prospective study was conducted during March 2010 to May 2011 after approval from Institutional Ethics Committee. Patients attending Dermatology, Medicine and Pediatrics. Out Patient Department (OPD) with suspected dermatophyte lesion of skin, hair or nail were included in the study. Clinical profile of the patients were recorded by taking a brief clinical history from the patient regarding occupation, history of living in institutions e.g. hostels, old age homes, children attending crèches etc., personal history regarding standard of hygiene, owning pets like cats or dogs, family history whether other family members were affected and past history regarding suffering from diabetes mellitus, eczema, tuberculosis, psoriasis, leprosy and also history of any form of treatment in the past.

The anatomical site (skin, hair and nail) in which the organism may be present was carefully selected. Specimens were collected aseptically in sufficient amount into sterile collection device or container before institution of antifungal agents and properly labeled. Specimen of skin was obtained by scraping at the active margin of lesion with a scalpel blade after cleaning the surface with 70% isopropyl alcohol. Infected nails were clipped by sterile nail trimmer. A portion of infected nail was scraped from the nail bed. Infected hair was epilated from the scalp with a sterile forceps.

Precautions were taken so that hair for culture was free from topical medications, conditioners and dressings.

10% or 20% KOH mount: A drop of 10% KOH was placed on a slide. The specimen was mixed with the drop and a cover slip was placed on it. The preparation was then passed over a flame for 2-3 times and examined under the microscope with 10x and 40x magnification to look for fungal elements. For nail scrapings and clippings 20% KOH was used.[4]

Culture: Each sample was inoculated on Dermatophyte Test Media (DTM) and Sabouraud Dextrose Agar (SDA) with and without antibiotics (cycloheximide and chloramphenicol) and incubated as per standard protocol. Both media were obtained from HiMedia Laboratories Pvt Ltd, Mumbai. The cultures were examined twice during the 1st week and weekly thereafter, for 4 weeks.

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Identification: Fungal growth was identified by gross colony morphology on SDA media, Lactophenol Cotton Blue (LPCB) mount, color change on DTM and microslide culture.

Clinical outcome of the patient could not be followed as all the subjects included in this study belonged to outpatient department and revisit by such patients is highly irregular.

STATISTICAL ANALYSIS: Statistical analysis was performed using Chi-square test. P <0.05 was considered significant.

RESULTS: A total of 372 samples were collected during the study period of which 141 samples showed growth of dermatophytes, 57 of Non dermatophyte, 36 contaminants while 138 samples showed no growth.

Among patients with skin, hair and nail infections majority of patients belonged to the age group 21-30 years and the overall male to female ratio was 2.6:1. Muslims were more frequently affected as compared to Hindus and the Muslim to Hindu ratio was 1.4:1. Maximum number of cases was seen in the month of June to September. In patients with dermatophytosis males were more commonly affected than females 102/141 (72.3%) vs. 39/141 (27.7%).

This finding was statistically significant (p= 0.000). Maximum numbers of dermatophytes were isolated from male patients in the age group of 21-30 years (35.3%) whereas in females maximum isolations were in the age group of 31-40 years (46.6%). The Muslim to Hindu ratio in cases of dermatophyte infection was 1.9:1, which was statistically significant (p=0.000) – [Table 1].

SDA with antibiotics supported the growth of 123/141 (87.2%) strains as compared to DTM, which showed growth of 114/141 (80.8%) strains. Direct microscopy with 10% or 20% KOH was less sensitive (46.8%) than culture on SDA with antibiotics (87.2%).

The most common dermatophyte isolated was Trichophyton rubrum 45/372 (12.1%) followed by Trichophyton tonsurans 36/372 (9.7%), Trichophyton mentagrophytes 30/372 (8.1%), Microsporum gypseum 18/372 (4.8%), Epidermophyton floccosum and Microsporum canis 6/372 (1.6%) each. Among non-dermatophyte species Aspergillus niger 21/372 (5.6%) was the commonest isolate followed by Aspergillus flavus and Rhizopus sp. 9/372 (2.4%) each. Aspergillus fumigatus, Penicillium sp., Fusarium sp., and Sepedonium sp. were isolated from 3/372 (0.8%) each. Candida sp. were isolated from 6/372 (1.6%) cases only – [Table 2].

Among clinical forms of dermatophytosis, majority of the patients had tinea corporis 78/141 (55.3%) followed by tinea cruris 18/141 (12.8%), tinea unguium and tinea pedis was seen in 15/141 (10.6%) each. In patients with tinea corporis Trichophyton rubrum was the most common organism 24/78 (30.8%) followed by Trichophyton tonsurans 21/78 (26.9%), Trichophyton mentagrophytes 12/78 (15.4%), Microsporum gypseum 9/78 (11.5%), Epidermophyton floccosum and Microsporum canis 6/78 (7.7%) each. In patients with tinea cruris, Trichophyton mentagrophytes was the commonest isolate 9/18 (50.0%) followed by Trichophyton tonsurans 6/18 (33.3%) and Trichophyton rubrum 3/18 (16.7%).

In tinea unguium Trichophyton rubrum and Trichophyton tonsurans were the commonest isolates 6/15 (40.0%) each whereas for tinea pedis Trichophyton mentagrophytes was the commonest 6/15 (40.0%). In tinea faciae and tinea capitis Trichophyton rubrum and Microsporum gypseum were isolated in 3/6 (50.0%) of cases. In all the 3 cases of tinea mannum only Trichophyton rubrum was isolated – [Table 3].

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DISCUSSION: On the basis of present study, the age distribution and sex distribution of dermatophytosis could be correlated with the studies conducted by other authors[5-9]. The Muslim to Hindu ratio of patient with dermatophytic infection was 1.9:1 which was statistically highly significant. The exact reason for this could not be ascertained as demographically speaking majority of patients from both groups' belonged to rural areas, were involved in agricultural work and also belonged to low socio-economic strata.

SDA with antibiotics was found to be much superior to DTM for culture of dermatophyte species. Culture also provide to be a better diagnostic tool when compared with microscopic examination with 10% or 20% KOH. The difference in the isolation of Trichophyton species as compared to others viz. Microsporum and Epidermophyton was found to be statistically significant (p= 0.000). Among non-dermatophyte species isolated Aspergillus species was the commonest, the other isolates being Rhizopus, Candida, Fusarium and Penicillium. Similar isolation patterns were also seen in other parts of India.[10, 11, 12]

The incidence of tinea corporis and the involvement of Trichophyton rubrum, which is the commonest isolate in this region, confirms the earlier findings regarding dermatopytosis in India.[5,13,14]

CONCLUSION: Dermatophytosis was found to be more prevalent in young adults (21-30 years). Culturing specimen on SDA with antibiotics was found to be the best method for diagnosis and this medium proved to be better than DTM. The most common dermatophyte was Trichophyton rubrum and the most common clinical form of dermatophytosis was tinea corporis. Fungal infections of the skin are not serious but their propensity to persist and lead to chronicity and disfigurement may have personal and social implications.

Though clinical diagnosis of these cases is easy enough for an experienced clinician or dermatologist, there is a lot of room for misdiagnosis as many of these skin lesions may mimic other skin disorders. This may in turn lead to unwarranted use of anti-fungal agents or use of steroids which may lead to flaring up of dermatophytic or non-dermatophytic fungal skin infections.

Diagnosis of these fungal infections in the laboratory needs to be encouraged not because of the fact that treating the patient requires so but because of the ease with which these organisms can be identified even in a laboratory with minimum basic infrastructure before initiating use of anti-fungal drugs. Microscopic examination also needs to be encouraged as reports can be generated very rapidly and may be helpful in preventing misuse of drugs. Culture on the other hand will help in ascertaining the changing scenario of the causative agents in a particular area.

Age (in years)	Male*		Female*	
	Hindu	Muslim	Hindu	Muslim
<10	1	2	0	0
11-20	7	11	2	1
21-30	11	25	3	9
31-40	8	16	7	11
41-50	5	4	2	4
51-60	1	5	0	0

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>60	2	4	0	0
Total	**35**	**67**	**14**	**25**

Table 1: Age and sex distribution of patients with dermatophytosis

(* p= 0.000)

Organism	Number of isolations	Percentage
*Trichophyton rubrum	45	12.1
*Trichophyton tonsurans	36	09.7
*Trichophyton mentagrophytes	30	08.1
*Microsporum gypseum	18	4.8
*Epidermophyton floccosum	6	1.6
*Microsporum canis	6	1.6
Aspergillus niger	21	5.6
Aspergillus flavus	9	2.4
Rhizopus sp.	9	2.4
Aspergillus fumigates	3	0.8
Penicillium sp.	3	0.8
Fusarium sp.	3	0.8
Sepedonium sp.	3	0.8
Candida sp.	6	1.6
Contamination	36	9.7
No growth	138	37.2
Total	**372**	**100.0**

Table 2: Overall distribution of organisms isolated from patients with skin, hair and nail infection

(* p= 0.000)

Dermatophyte isolated	Clinical forms						
	Tinea corporis (%)	Tinea cruris (%)	Tinea unguium (%)	Tinea pedis (%)	Tinea faciae (%)	Tinea capitis (%)	Tinea mannum (%)
Trichophyton rubrum	24 (30.8)	3 (16.7)	6 (40.0)	3 (20.0)	3 (50.0)	3 (50.0)	3 (100.0)
Trichophyton tonsurans	21 (26.9)	6 (33.3)	6 (40.0)	3 (20.0)	0	0	0
Trichophyton mentagrophytes	12 (15.4)	9 (50.0)	3 (20.0)	6 (40.0)	0	0	0
Microsporum gypseum	9 (11.5)	0	0	3 (20.0)	3 (50.0)	3 (50.0)	0
Epidermophyton floccosum	6 (7.7)	0	0	0	0	0	0
Microsporum canis	6 (7.7)	0	0	0	0	0	0
Total	**78 (100.0)**	**18 (100.0)**	**15 (100.0)**	**15 (100.0)**	**6 (100.0)**	**6 (100.0)**	**3 (100.0)**

Table 3: Distribution of clinical forms of dermatophytosis

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REFERENCES:

1. Rook A, Wilkinson DS, Ebling FJG editors. Text Book of Dermatology. vol 2, 4th ed. United Kingdom: Blackwell Scientific Publications, Oxford; 1988.p.893-927.
2. Padhye AA and Summerbell RC. The dermatophytes. In: Merz WG, Hay RJ editors. Topley & Wilson's Medical Mycology. 10th ed. London: Hodder Arnold ASM press; 2005.223-37.
3. Chander J editor. Dermatophytoses. In: Textbook of Medical Mycology. 3rd ed. New Delhi: Mehta publishers; 2009.122-46.
4. Milney LJR. Fungi. In: Collee JG, Fraser AG, Marmiom BP, Simmons A, editors. In: Mackie and Mc-Cartney Practical Medical Microbiology. 14th ed. New Delhi: Churchill Livingstone; 2008.697-8.
5. Mohanty JC, Mohanty SK, Sahoo RC, Sahoo A, Praharaj N. Incidence of dermatophytosis in Orissa. Indian J Med Microbiol 1998; 16 (2): 78-80.
6. Mulay DN, Ahuja BB and Garg AK. A study on the Ecology and treatment of Dermatophytosis in Delhi. Indian J Dermatol Venereol Leprol 1970; 36 (6): 215-20.
7. Sumana MN and Rajagopal V. A clinic-epidemiological study of dermatophytosis in North East India. Indian J Pathol Microbiol 2002; 45 (2): 169-72.
8. Das NK, Ghosh P, Das S, Bhattacharya S, Dutta RN, Sengupta R. A study of etiological agents and clinicomycological correlation of fingernail onychomycosis in Eastern India. Indian J Dermatol 2008; 53 (2): 75-9.
9. Sahai S and Mishra D. Change in spectrum of dermatophytes isolated from superficial mycoses cases: First report from Central India. Indian J Dermatol Venereol Leprol 2011; 77 (3): 335-6.
10. Bindu V and Pavitran K. Clinico-mycological study of dermatophytosis in Calicut. Indian J Dermatol Venereol Leprol 2002; 68 (5): 259-61.
11. Peerapur BV, Inamdar AC, Pushpa PV, Srikant B. Clinicomycological study of Dermatophytosis in Bijapur. Indian J Med Microbiol 2004; 22 (4): 273-4.
12. Sarma S and Borthakur AK. A clinico-epidemiological study of dermatophytoses in Northeast India. Indian J Dermatol Venereol Leprol 2007; 73 (6): 427-8.
13. Padhye AA, Thirumalachar HJ and Gokhale BB. Dermatophytosis in Poona, India, Observation on incidence, clinical features, environmental factors and causal agents during 1958-63 at Sassoon hospital, Poona. Mycopathologia 1968; 40 (3): 325-36.
14. Mulay DN, Ahuja BB and Garg AK. A study on the Ecology and Treatment of Dermatophytosis in Delhi. Indian J Dermatol Venereol Leprol 1970; 36 (6): 215-20.

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ORIGINAL ARTICLE

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METHICILLIN AND VANCOMYCIN RESISTANCE AMONG STAPHYLOCOCCUS AUREUS STRAINS ISOLATED FROM PATIENTS ATTENDING TERTIARY CARE HOSPITAL IN EASTERN BIHAR

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ABSTRACT

BACKGROUND

Staphylococcus aureus is notorious for its ability to become resistant to antibiotics. MRSA emerged as nosocomial pathogen in the early 1960s. Methicillin Resistant Staphylococcus aureus (MRSA) are implicated in serious infections and nosocomial infection outbreaks. These strains show resistance to a wide range of antibiotics, thus limiting the treating options to very few agents such as vancomycin and teicoplanin. Vancomycin has been regarded as the first-line drug for treatment of MRSA. At the same time, there has been an increase in the use of this antibiotic for other infections as well. This has further lead to an increase in the number of both Vancomycin Intermediate Staphylococcus aureus (VISA) and Vancomycin Resistant Staphylococcus aureus (VRSA).
Aims- To determine the presence of MRSA and VRSA among staphylococcal isolates in Eastern Bihar.

MATERIALS AND METHODS

A total of 10806 samples from patients attending inpatient and outpatient departments from January 2011 to April 2013 were included in the study. Samples were processed as per standard protocol and antibiotic susceptibility testing was done by modified Kirby–Bauer method. Isolates were tested by disc diffusion using oxacillin disc 1 µg, cefoxitin disc 30 µg and by agar dilution for MRSA. VRSA isolates were tested using 30 µg vancomycin disc. MIC of vancomycin to Staphylococcus aureus was determined by agar dilution method.

RESULTS

Out of a total of 633 Staphylococcus aureus isolates, 22.4% were found to be methicillin resistant, 9.95% were VISA and 3.79% were VRSA. Results of oxacillin agar dilution method were in concordance with the cefoxitin disc diffusion method in detecting MRSA strains. All VISA strains were sensitive to linezolid and all VRSA were sensitive to imipenem. 88.7% and 87.3% of all MRSA isolates were sensitive to imipenem and linezolid respectively.

CONCLUSION

The present shows that antibiotic resistance is steadily on the rise. It is also quite clear that MRSA is acquiring resistance to drugs like rifampicin, teicoplanin, amikacin, netilmicin and imipenem which were at one time used as an alternative to vancomycin. It is therefore imperative for the medical community to work together to fight against this man-made phenomenon called antibiotic resistance.

KEYWORDS

MRSA, VISA, VRSA.

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BACKGROUND

Methicillin Resistant Staphylococcus aureus (MRSA) are implicated in serious infections and nosocomial infection outbreaks. These strains show resistance to a wide range of antibiotics (multi-resistance), thus limiting the treating options to very few agents such as vancomycin and teicoplanin. Therefore, it is clinically crucial to determine rapidly whether Staphylococcus aureus isolates are methicillin resistant or not and this is of utmost importance for both treatment and control as it requires extensive hygienic precautions to limit the spread of such strains.[1]

Staphylococcus aureus is notorious for its ability to become resistant to antibiotics. Infections that are caused by antibiotic-resistant strains often occur in endemic waves that are initiated by one or a few successful clones. MRSA features prominently in these epidemics. Historically associated with hospital and other health care settings, MRSA has now emerged as a widespread cause of community infections as well. Community-associated MRSA (CA-MRSA) can spread rapidly among healthy individuals. Outbreaks of CA-MRSA infection has been reported worldwide.[2]

The frequency of MRSA infections continues to grow in hospital-associated settings, and more recently in community setting globally. The increase in the incidence of infections due to Staphylococcus aureus is partially a consequence of

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advances in patients care and also of the pathogen's ability to adapt to a changing environment. Infection due to Staphylococcus aureus imposes a high and increasing burden on health care resources. A growing concern is the emergence of MRSA infection in patients with no apparent risk factors. In India MRSA prevalence has increased from 12% in 1992 to 80.83% in 1999.[2]

MRSA emerged as nosocomial pathogen in the early 1960s. Most occurrences were isolated occurring in sporadic outbreaks but in 1970s, an increasing number of large hospital outbreaks were reported in many countries including USA, Europe, Japan, and Australia. Staphylococcus aureus is the predominant organism isolated from surgical wound infections with a prevalence rate ranging from 4.6%-54.4% of all Staphylococcus aureus isolations.[3]

Most of these nosocomial infections include bacteraemia, surgical wound infections and pneumonia as well. The problem of MRSA infection is exacerbated by the propensity of the organism to cause cross-infection and its ability to colonize individuals for months or years. Selection pressure for this organism is seen in hospital settings due to intensive use of many antibiotics especially cephalosporins, to which it is resistant.[4]

Vancomycin has been regarded as the first-line drug for treatment of MRSA. At the same time, there has been an increase in the use of this antibiotic for other infections as well such as pseudomembranous colitis due to Clostridium difficile and coagulase negative staphylococcal infections in hospitalized patients. When the drug was introduced, it was believed that there would be no resistance to this drug as resistance was difficult to induce. In 1997 however, the first strain of Staphylococcus aureus with reduced susceptibility to vancomycin was reported from Japan. Since then there has been an increase in the number of both vancomycin intermediate Staphylococcus aureus (VISA) and vancomycin resistant Staphylococcus aureus (VRSA). This has led to life threatening infections in both hospitalized and non-hospitalized patients.[4]

Taking into consideration the menace that is MRSA both in hospital and more recently in community settings and also the emergence of VISA and VRSA the study was undertaken to determine the presence of MRSA and VRSA among staphylococcal isolates in Eastern Bihar.

MATERIALS AND METHODS

This prospective study was conducted from January 2011 to April 2013 with approval from Institutional Ethics Committee. Patients attending various in-patient and out-patient departments of the hospital were inducted into the study. A brief clinical history was obtained from the patient regarding occupation, history of living in institutions e.g. hostels, old age homes etc., personal history regarding standard of hygiene, family history whether other family members were affected and past history.

Urine, pus, blood and vaginal specimens were collected as per standard protocol. Samples were processed and identified as per standard protocol.[5] Antibiotic sensitivity testing was done by modified Kirby-Bauer's disc diffusion test as per the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2008). Commercially available antibiotic discs (hi-Media) were used. Screening for MRSA was done by disc diffusion and agar dilution method.

Screening for MRSA by Disc Diffusion

The test was performed on Muller-Hinton agar with 4% NaCl for oxacillin and without 4% NaCl for cefoxitin. Using the same procedure as for AST oxacillin disc 1 µg and cefoxitin disc 30 µg were used and plates were incubated at 35°C for 24 hrs. Results were interpreted as per manufacturer interpretation chart. ATCC 25923 was used as sensitive control.[6]

Screening of MRSA by Agar Dilution

Oxacillin powder was obtained in pure form from Hi-Media laboratories, Mumbai. Commercially available dehydrated Muller-Hinton agar from the same company was used to prepare Muller-Hinton agar to which NaCl was added to a final concentration of 4%. Amount of antibiotic powder to be added to this medium to make a final concentration of 6 µgm/ml oxacillin was calculated by the following formula:

$$\frac{1000}{P} \times V \times C = W$$

Where

P= potency of preparation in relation to base.
V= volume (ml) required.
C= final concentration of solution in multiples of 1000.
W= weight in mg of antibiotic to be dissolved in V (volume).[7]

Colonies of the test organism were suspended in 4 ml of sterile normal saline to adjust the turbidity to 0.5 McFarland's standard. The bacterial strains were spot inoculated on the surface of Mueller-Hinton agar containing 4% NaCl and 6 µgm/ml oxacillin using 10 µl of bacterial culture suspension. When the inoculum dried up the plates were incubated at 35°C for 24 hrs.

Interpretation: No Growth - Oxacillin susceptible.
Growth - Oxacillin resistant.[6]

Screening for VRSA by Disc Diffusion

The test was performed on plain Mueller-Hinton agar using 30 µg vancomycin disc using the modified Kirby-Bauer's diffusion method. Results were interpreted as per manufacturer interpretation chart. ATCC 25923 was used as sensitive control.[6] (CLSI, 2008).

MIC of VRSA by Agar Dilution

Vancomycin powder was obtained in pure form from Hi-Media laboratories, Mumbai. Minimum Inhibitory Concentration (MIC) of vancomycin to Staphylococcus aureus was determined by agar dilution method. The gradient plates of Mueller-Hinton agar were prepared with different concentrations of vancomycin (i.e. 1, 2, 4, 8, 16, 32 and 64 µg/ml). The test organisms were suspended in sterile normal saline and the turbidity was matched to 0.5 McFarland's standard. The bacterial strains were spot inoculated on the surface of Mueller-Hinton agar using 10 µl of bacterial culture suspension. When the inoculum dried up the plates were incubated at 35°C for 24 hrs.

Interpretation: MIC ≤ 2 µg/ml- Susceptible.
MIC 4-8 µg/ml- Intermediate Susceptible.
MIC ≥16 µg/ml- Resistant.[6]

RESULTS

A total of ten thousand eight hundred and six (10806) samples were collected during the study period of which 4482 samples showed growth of various microorganisms. Staphylococcus species grew in 873 samples out of which 240 were Coagulase Negative Staphylococcus (CONS) and 633 were Staphylococcus aureus. These 633 strains of Staphylococcus aureus were taken up for further study.Out of a total of 633 Staphylococcus aureus isolates, 142 (22.4%) were found to be methicillin resistant, 63 (9.95%) were VISA and 24 (3.79%) were VRSA. Majority of MRSA and VRSA strains were isolated from the age group 21-30 years, 47/142 (33.1%) and 13/24 (54.2%) respectively. MRSA and VRSA infections were seen predominantly in males 79/142 (55.6%) and 15/24 (62.5%) respectively. The male to female ratio for MRSA infection was 1.25:1 and for VRSA 1.66:1. MRSA was isolated more frequently from patients of the Hindu community, 87/142 (61.3%) whereas VRSA were isolated more frequently from the Muslim community 14/24 (58.5%). The Hindu to Muslim ratio for MRSA infection was 1.58:1 and for VRSA was 1:1.66.

Most of the MRSA and VRSA were isolated from pus samples followed by urine Table 1. Overall 33.1% (47/142) of MRSA and 42.8% (27/63) of VISA were isolated from Surgery Department whereas 41.7% (10/24) of VRSA was isolated from Orthopaedics Department. Majority of MRSA (88.7%), VISA (74.6%) and all VRSA were isolated from in-patients department.

Results of oxacillin agar dilution method were in concordance with the cefoxitin disc diffusion method detecting 22.4% (142/633) of strains as MRSA. The oxacillin disc diffusion method however, detected sixteen additional strains thereby increasing the number of MRSA detected by this method to 25.0% (158/633). Vancomycin DD test detected only 14 strains of VRSA out of a total of 633 strains. The Vancomycin agar dilution test however showed that 24 strains had MIC ≥ 16 µg/ml indicating resistance (VRSA) and 63 strains had MIC ranging from 4-8 µg/ml indicating intermediate resistance (VISA). Table 2.

As with MRSA, VISA and VRSA strain, it showed 100% resistance to ampicillin, cefuroxime and cefotaxime. 73% and 75% of VISA and VRSA were resistant to gentamicin. All VISA strains were sensitive to linezolid and all VRSA were sensitive to imipenem. 88.7% and 87.3% of all MRSA isolates were sensitive to imipenem and linezolid respectively. Table 3.

Specimen	MRSA (%)	VISA (%)	VRSA (%)
Pus	79 (55.7)	34 (54.0)	14 (58.3)
Urine	47 (33.1)	18 (28.6)	07 (29.2)
Blood	08 (5.6)	07 (11.1)	02 (8.3)
Vaginal Swab	08 (5.6)	04 (6.3)	01 (4.2)
Total	**142 (100)**	**63 (100)**	**24 (100)**

Table 1. Isolation of MRSA, VISA and VRSA from Different Clinical Samples

Method	Resistant (%)	Intermediate (%)	Sensitive (%)	Total
Vancomycin DD	14 (2.2)	0 (0.0)	619 (97.8)	633
Vancomycin MIC	24 (3.7)	63 (10.0)	546 (86.3)	633

Table 2. Correlation between different methods for detection of VRSA

Antibiotics (Potency)	MRSA (%) n=142		VISA (%) n=63		VRSA (%) n=24	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Amikacin (30 µgm)	110 (77.5)	32 (22.5)	57 (90.5)	06 (9.5)	18 (75.0)	06 (25.0)
Ampicillin (10 µgm)	00(0.0)	142 (100.0)	00 (0.0)	63 (100.0)	00 (0.0)	24 (100.0)
Cefotaxime (30 µgm)	00 (0.0)	142 (100.0)	00 (0.0)	63 (100.0)	00 (0.0)	24 (100.0)
Cefuroxime (30 µgm)	00 (0.0)	142 (100.0)	00 (0.0)	63 (100.0)	00 (0.0)	24 (100.0)
Clindamycin (2 µgm)	87 (61.3)	55 (38.7)	46(73.0)	17 (27.0)	18 (75.0)	06 (25.0)
Gentamicin (10 µgm)	63 (44.4)	79 (55.6)	17 (27.0)	46 (73.0)	06 (25.0)	18 (75.0)
Imipenem (10 µgm)	126 (88.7)	16 (11.3)	57 (90.5)	06 (9.5)	24 (100.0)	00 (0.0)
Linezolid (30 µgm)	124 (87.3)	18 (12.7)	63 (100.0)	00 (0.0)	12 (50.0)	12 (50.0)
Teicoplanin (30 µgm)	71 (50.0)	71 (50.0)	11 (17.5)	52 (82.5)	00 (0.0)	24 (100.0)
Tobramycin (10 µgm)	89(62.7)	53 (37.3)	46 (73.0)	17 (27.0)	12 (50.0)	12 (50.0)

Table 3. Antibiotic Susceptibility Pattern for MRSA, VISA and VRSA

DISCUSSION

The present study gives us an indication regarding the occurrence of MRSA, VISA and VRSA in this region. As far as MRSA is concerned different studies have reported different findings. Authors have reported 79.6% of MRSA from Hyderabad,[8] 34.2% from South India.[9] The MRSA isolation in our region was found to be relatively low (22.50% of all Staphylococcus aureus isolates) as compared to the isolation rates in some other part of India, which is probably because the Medical College is situated in rural area where the organisms are not exposed to as much antibiotic pressure as in the urban areas. For VISA and VRSA Thati et al[8] 2011 reported 4.47% isolation of VISA and 1.96 which is lower than ours.

Majority of the MRSA, VISA and VRSA strains were isolated from Surgery department followed by Obstetrics & Gynaecology and orthopaedics. Tyagi A et al[3] in 2008 however, reported maximum isolations from Neurosurgery ward followed by Orthopaedics, Paediatric Surgery, and Cardiothoracic Surgery etc.[3] This discrepancy may be due to the fact that their study was carried out in a super-specialty hospital and also in a different geographic location.

Isolation rate of MRSA, VISA and VRSA in the present study were much higher in indoor patients, as hospital strains are subjected to more antibiotic pressure leading to

emergence of resistant strains. Majority of MRSA, VISA and VRSA were also isolated from pus samples followed by urine samples and blood and vaginal swabs as reported by other authors also[10,11] The oxacillin disc diffusion method however, detected sixteen additional strains. This is probably due to the fact that oxacillin DD test may sometime produce smaller zone of inhibition for those strains of Staphylococcus aureus that are hyperproducers of penicillinase enzymes.[12] These sixteen strains were therefore not considered as MRSA.

Kour R et al in 2012 have also reported a similar finding in their study on MRSA[13]. They found the oxacillin DD to be inferior to the cefoxitin DD for detection of MRSA. It is quite apparent from all the studies conducted so far and from the present study that antibiotic resistance is steadily on the rise. It is also quite clear that MRSA is acquiring resistance to drugs like rifampicin, teicoplanin, amikacin, netilmicin and imipenem which were at one time used as an alternative to vancomycin, which is more toxic and has to be delivered parenterally, for treating MRSA infection.[2,3,10,14] It is very clear from the present study that resistance to antibiotics is high even in Methicillin Sensitive Staphylococcus aureus (MSSA) though lesser to a certain extent than MRSA. Antibiotic resistance remains a problem even in this rural area where majority of the population are employed in agricultural work. It is therefore imperative for the medical community to work together to fight against this man-made phenomenon called antibiotic resistance.

REFERENCES

[1] Araj GF, Talhauk RS, Simman CJ, et al. Discrepancies between mecA PCR and conventional tests used for methicillin resistant staphylococcus aureus. Int J Antimicrobial agents 1999;11(1):47-52.

[2] Tsering DC, Pal R, Kar S. Methicillin resistant staphylococcus aureus: prevalance and current susceptibility pattern in sikkim. Journal of Global Infectious Disease 2011;3(1):9-13.

[3] Tyagi A, Kapil A, Singh P. Incidence of methicillin resistant staphylococcus aureus (MRSA) in pus samples at a tertiary care hospital, AIIMS, New Delhi. Journal Indian Academy of Clinical Medicine 2008;9(1):33-5.

[4] Sood PL, Taneja J, Mishra B. Methicillin and vancomycin resistant staphylococcus aureus in hospitalized patients. Journal of Global Infectious Disease 2010;2(3):275-83.

[5] Collee JG, Marr W. Culture of bacteria. In: Collee JG, Fraser AG, Marmion BP, et al. (eds) Mackie and McCartney practical medical microbiology. 14th edn. Churchill Livingstone: New Delhi 2006:113-29.

[6] CLSI. Performance standards for antimicrobial susceptibility testing: eighteenth international supplements, M100-S18 2008;28(1):1-179.

[7] Miles RS, Amyes SGB. Laboratory control of antimicrobial therapy. In: Collee JG, Fraser AG, Marmion BP, et al. (eds) Mackie and McCartney practical medical microbiology. 14th edn. Churchill Livingstone: New Delhi 2006:151-78.

[8] Thati V, Shivannavar CT, Gaddad SM. Vancomycin resistance among methicillin resistant staphylococcus aureus isolates from intensive care units of tertiary care hospitals in Hyderabad. Indian Journal of Medical Research 2011;134(5):704-8.

[9] Dhanalakshmi TA, Umapathy BL, Mohan DR. Prevalence of methicillin, vancomycin and multidrug resistance among staphylococcus aureus. Journal of Clinical and Diagnostic Research 2012;6(6):974-7.

[10] Anupurva S, Sen MR, Nath G, et al. Prevalence of methicillin resistant staphylococcus aureus in tertiary referral hospital in eastern Uttar Pradesh. Indian Journal of Medical Microbiology 2003;21(1):49-51.

[11] Sharma P, Vishwanath G. Study of vancomycin susceptibility in methicillin-resistant staphylococcus aureus isolated from clinical samples. Annals of Tropical Medicine and Public Health 2012;5(3):178-80.

[12] Peacock SJ. Staphylococcus. In: Borrello PS, Murray PR, Funke G (eds). Topley & Wilsons's microbiology & microbial infections. 10th edn. Hodder Arnold, London 2005:771-832.

[13] Kaur R, Oberoi L, Aggrawal A. Comparative evaluation of latex agglutination method and other phenotypic method for detection of methicillin-resistant staphylococcus aureus. Indian Journal of Medical Microbiology 2012;30(2):252-3.

[14] Rajaduraipandi K, Mani KR, Panneerselvam K, et al. Prevalence and antimicrobial susceptibility pattern of methicillin resistant staphylococcus aureus: a multicentre study. Indian Journal of Medical Microbiology 2006;24(1):34-8.

ORIGINAL RESEARCH PAPER
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A CLINICAL AND BACTERIOLOGICAL STUDY OF NEONATAL SEPTICAEMIA IN KATIHAR MEDICAL COLLEGE & HOSPITAL, KATIHAR, BIHAR.

Microbiology

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ABSTRACT

Background: Neonatal sepsis is a clinical syndrome of bacteraemia characterized by systemic signs and symptoms inthe first month of life. It is the leading causes of neonatal mortality and morbidity. Early diagnosis and treatment with appropriate antibiotics is important to improve the prognosis of neonatal sepsis. Our objectives were to study the organisms causing neonatal septicaemia, associated risk factors, to correlate CRP with blood culture and to study mortality rate in neonatal septicaemia. **Methods:** The study of one year included clinically suspected cases of neonatal septicaemia admitted in NICU (KMCH, Katihar, Bihar). 566blood samples were collected, processed and isolates were identified. Maternal and neonatal risk factors were studied. CRP test was done by slide agglutination test. **Results:** Blood culture was positive in 205 (36.22%) cases. Among the culture positive cases, 128 (62.44%) weremales and 77 (37.56%) females with male to female ratio of 1.66:1. Early onset sepsis was present in 137 (66.83%) and late onset sepsis in 68 (33.17%) cases. 107 (52.20%) were low birth weight babies. The most common neonatal risk factor was prematurity 75 (36.58%) and maternal risk factor was prolonged rupture of membrane 65 (31.71%). gram negative bacilli 144 (70.24%) were found to be common cause of sepsis than gram positive cocci 61 (29.76%), Klebsiellapneumoniae54 (26.34%) being most common pathogen. Out of 566, CRP test was positive in 244(43.10%) cases. Mortality rate was 23.41%. **Conclusions:** Neonatal septicaemia is a life-threatening emergency. The study of etiological profile and CRP testplays a significant role.

KEYWORDS

INTRODUCTION

Neonatal sepsis is a clinical syndrome of systemic illness accompanied by bacteremia occurring in the first month of life. The immaturity of immune system in the neonates makes them especially susceptible to infections during the neonatal and perinatal period. Neonatal septicaemia is by far the most important and often fatal sequelae of such infection. In India it is commonest cause of neonatal mortality contributing to 38% of the neonatal deaths. For epidemiological and therapeutic purposes, neonatal septicaemia is categorised into early onset neonatal septicaemia (EONS), which presents within the first 72 hours of life and late onset neonatal septicaemia (LONS) presenting after 72 hours of life.[1] This distinction has clinical relevance, as early onset neonatal sepsis is generally acquired from pathogens of maternal genital tract, whereas late onset sepsis has its origin either from the community or from hospital.

In neonatal sepsis numerous neonatal, maternal and environmental risk factors contribute to the high morbidity and mortality. The various risk factors which are associated with EONS are low birth weight (<2500gms), preterm baby, febrile illness in the mother, foul smelling and/or meconium stained liquor amnii, prolonged rupture of membrane (>24 hours), more than 3 vaginal examinations during labor, prolonged and difficult delivery with instrumentation, perinatal asphyxia or difficult resuscitation. Whereas the risk factors for development of LONS include prolonged NICU admission, poor hygiene, low birth weight (LBW), poor cord care, prematurity, bottle feeding, invasive procedure, superficial infection (pyoderma, umbilical sepsis), prelacteal feeding, ventilation, aspiration of feeds etc.

Early diagnosis is a key to reduce morbidity and mortality of neonatal septicaemia. The gold standard for diagnosis of septicaemia is the isolation of bacterial agents from the blood culture. But definitive culture results take at least 48-72 hours resulting in treatment delays. Hence two-pronged approach is used for the evaluation of neonates with possible sepsis. Nonspecific sepsis screen tests like C-reactive protein (CRP), erythrocyte sedimentation rate, total white blood cell count and absolute neutrophil count are used to evaluate the likelihood of infection, and specific diagnostic tests are performed to confirm the presence of a specific pathogen in body fluids.

Both gram negative and gram-positive bacteria have been isolated from blood. Organisms causing sepsis and their susceptibility to different antibiotics vary from place to place. As neonatal septicaemia is life threatening emergency, early diagnosis and treatment with appropriate antibiotics is necessary. Study objective was to study the organisms causing neonatal septicaemia in our region, risk factors associated with neonatal septicaemia, to correlate CRP with blood culture and to study the mortality rate in neonatal septicaemia.

METHODS

The observational study of one year was carried out from May 2018 to April 2019, in the Department of Microbiology, Katihar Medical College, Katihar, Bihar. Clinically suspected cases of neonatal septicaemia admitted in neonatal intensive care unit (NICU) were included in the study. Prior to collection of blood sample, consent from mother was taken and detailed history of each neonate along with history of maternal risk factors, neonatal risk factors and mode of delivery etc., was recorded.

Blood culture

The skin of venepuncture site was disinfected with 70% alcohol and 1% iodine for at least 1 minute and allowed to dry. With precaution to avoid touching and contaminating venepuncture site, 2 ml of blood was withdrawn with disposable needle and syringe and inoculated into blood culture bottle containing 20 ml of trypticase soy broth. The blood and broth were mixed gently; the bottles were transported immediately to laboratory and incubated at 37°C aerobically. First subculture was done after 6-17 hours on 5% sheep blood agar and Mac Conkey agar plates. Thereafter daily subculture was done for 7 days. The isolates were identified by Gram staining, colony characteristics and biochemical properties. Cultures were labelled negative if there was no growth after 1 week of incubation. Antimicrobial susceptibility of all bacterial isolates was done by Kirby-Bauer disk diffusion technique as per CLSI 2014 guidelines.

C-Reactive protein (CRP) test

For CRP testing, 2 ml Blood samples was collected and serum was separated. Testing was done by slide agglutination test according to manufacturer's instructions (Tulip diagnostics (P) Ltd).

Statistical analysis

Statistical analysis was done by chi square test and Mc-Nemar Chi-square test. p value <0.05 was considered as statistically significant.

RESULTS

Out of total 566 blood samples subjected for culture, 205 (36.22%) were culture positive and 361 (63.78%) were culture negative. The

culture positivity rate was 36.22%. Out of total 205 culture positive cases 137 (66.83%) were of age less than 3 days belonging to early onset septicaemia, while 68 (33.17%) cases were between the age of 3 days to 28 days belonging to late onset septicaemia (Table 1). Among them 128 (62.44%) were males and 77 (37.56%) were females with male to female ratio of 1.66:1.

Table 1: Age wise distribution of culture positive cases (n=205).

Age (days)	No. of cases	Total (%)
0-3	137	137 (66.83) EONS
4-6	8	68 (33.17) LONS
7-9	4	
10-12	12	
13-15	5	
16-18	12	
19-21	8	
22-24	10	
25-28	9	
0-28	205	205 (100)

Table 2 shows birth weight wise distribution of the cases. Out of the total 205 culture positive neonates, maximum neonates 107 (52.20%) were low birth weight babies. The most common neonatal risk factor responsible for the infection was prematurity in 75 (36.58%) neonates, followed by respiratory distress in 70 (34.15%) neonates (Table 3).

Table 2: Birth weight wise distribution of culture positive cases (n=205).

Neonatal Birth Weight	No. of culture positive (%)
Normal birth weight (≥2500g)	53 (25.85)
Low birth weight (<2500g)	107 (52.20)
Very low birth weight (<1500g)	45 (21.95)
Extremely low birth weight (<1000g)	0
Total	205 (100)

Table 3: Neonatal risk factors among culture positive cases (n=205).

Risk Factors	Culturepositive cases (%)
Prematurity	75 (36.58)
Respiratory Distress	70 (34.15)
Parenteral Nutrition	57 (27.80)
Mechanical Ventilation	52 (25.36)
Birth Asphyxia	07 (03.36)

Table 4 shows maternal risk factors. Mothers of 65 (31.71%) neonates had history of prolonged rupture of membrane (PROM) and maternal fever was seen in 23 (11.22%). Of the total 205 culture positive neonates, total 105 (51.22%) were delivered in hospital while 15 (07.31%) were delivered at home by normal vaginal delivery. Lower segment caesarean section (LSCS) was the mode of delivery in 65 (31.71%) cases and instrumentation was used in mothers of 20 (09.76%) neonates (Table 5).

Table 4: Maternal risk factors among culture positive cases (n=205).

Risk Factors	Culturepositive cases (%)
Prolonged Rupture of Membrane	65 (31.71)
Maternal Fever	23 (11.22)

Table 5: Distribution of culture positive cases as per mode of delivery (n=205).

Mode and place of delivery	Culturepositive cases (%)
Normal vaginal delivery at hospital	105 (51.22)
Normal vaginal delivery at home	15 (07.31)
Lower segment caesarian section (LSCS)	65 (31.71)
Instrumentation	20 (09.76)
Total	205 (100)

Table 6 shows the isolates from blood culture of neonatal septicaemia cases. Gram negative bacilli 144 (70.24%) were common etiological agents as compared to gram positive cocci 61 (29.76%). The most common gram-negative organism causing sepsis was *Klebsiella pneumoniae* 54 (26.34%) and gram-positive organism was S. aureus 36 (17.56%).

Out of total 137 EONS cases, gram negative bacilli were 103 (75.18%) and gram-positive cocci were 34 (24.82%). Of them, Klebsiella pneumoniae 44 (32.12%) was the commonest isolate. In LONS cases (68), gram negative bacilli 41 (60.29%) predominated as compared to gram positive cocci 27 (39.71%) and the most common pathogen was *S. aureus 22* (32.35%).

Table 6: Distribution of organisms as per the onset of septicaemia.

Organisms Isolated	EONS (%)	LONS (%)	Total
Gram negative organisms	103 (75.18)	41 (60.29)	144 (70.24)
Klebsiella pneumonia	44 (32.12)	10 (14.71)	54 (26.34)
Pseudomonas aeruginosa	11 (08.03)	16 (23.53)	27 (13.17)
Escherichia coli	20 (14.59)	03 (04.41)	23 (11.22)
Acinetobacterbaumannii	04 (02.92)	10 (14.71)	14 (06.83)
Enterobacterspp	08 (05.84)	00(0)	08 (03.90)
Citrobacterspp	08 (05.84)	00 (0)	08 (03.90)
Klebsiellaoxytoca	06 (04.38)	00 (0)	06 (02.93)
Acinetobacterlwoffii	02 (01.46)	02 (02.94)	04 (01.95)
Gram positive organisms	34 (24.82)	27 (39.71)	61 (29.76)
Staphylococcus aureus	14 (10.22)	22 (32.35)	36 (17.56)
CONS	14 (10.22)	04 (05.88)	18 (08.78)
Enterococcus faecalis	04 (02.92)	01 (01.47)	05 (02.44)
Streptococcus pneumonia	02 (01.46)	00 (0)	02 (00.98)
Total	137 (100)	68 (100)	205 (100)

Selection of antibiotics and study of sensitivity pattern of the isolates was done as per CLSI guidelines.[9] The most predominant isolate *Klebsiella pneumoniae* was 54 (100%) resistant to Ampicillin and Cefazolin, while they were sensitive to Imipenem 52 (96.30%) and Amikacin 45 (83.34%). All 27 (100%) isolates of *P. aeruginosa* were sensitive to Polymyxin B and *Colistin. Acinetobacter spp* (n=18) showed least resistance to Imipenem (27.78%) and Amikacin (33.33%).

Amongst gram positive organisms, *S. aureus* showed resistance to Penicillin, Cefoxitin, Erythromycin, Gentamycin and Amikacin (94.44%, 52.78%, 41.67%, 33.33% and 16.67% respectively). 52.78% of the Staphylococcus aureus were methicillin-resistant *S. aureus* (MRSA). All the 36 isolates of *S. aureus* and 18 isolates of CONS were 100% sensitive to Vancomycin and Linezolid. Among *Enterococcus faecalis* (n=05), maximum resistance of 60% was seen to Penicillin and Ampicillin and were 100% sensitive to high level Gentamycin, Vancomycin and Linezolid.

CRP test was performed in all 566 clinically suspected cases of septicaemia and its correlation with blood culture was studied. Out of 566 cases, CRP was positive in 244 (43.10%) and negative in 322 (56.90%) cases. Table 7 shows, out of 205 culture positive cases, 198(96.59%) were CRP positive and 07 (3.41%) were CRP negative while out of 361 culture negative cases, 46 (12.74%) were CRP positive and 315 (87.26%) were CRP negative, with a significant p value. Sensitivity and specificity of the CRP test was 96.59% and 87.26% respectively.

Table 7: Correlation of CRP with blood culture positivity (n=566).

CRP Test	Blood Culture		Total
	Culture Positive	Culture Negative	
Positive	198 (96.59%)	46 (12.74%)	244
Negative	07 (3.41%)	315 (87.26%)	322
Total	205 (100%)	361 (100%)	566

Mc-Nemar Chi square test used, p=8.46×10-8 (p=0.000).

Table 8 shows the mortality of neonates with respect to onset of septicaemia. Out of 137 EONS cases 35 (25.54%) and 68 LONS cases 13 (19.11%) were died of septicaemia. Overall mortality rate was 23.41%. The statistical difference in the mortality rates between two types of septicaemia was not significant.

Table 8: Mortality of culture positive cases as per the onset.

Onset	Culture Positive Cases	Mortality (%)
Early onset neonatal septicemia	137	35 (25.54)
Late onset neonatal septicemia	68	13 (19.11)
Total	205	48 (23.41)

CONCLUSION

Gram negative bacilli were found to be commonest cause of neonatal septicaemia in our setup. Male neonates were more prone to infection.

Incidence of EONS was common as compared to LONS. Various neonatal and maternal risk factors were found to be associated with neonatal septicaemia. The prematurity and low birth weight neonates were at an increased risk of developing sepsis. Blood culture should be done in all suspected cases. The most common pathogen isolated in EONS cases was *K. pneumoniae* and in LONS cases was *S. aureus*. A good correlation between blood culture positivity and CRP was found in the present study. So, CRP testing may prove helpful marker to improve diagnostic accuracy in resource limited situations. Neonatal septicaemia is important cause of morbidity and mortality among the neonates. Early diagnosis, specific treatment and strict infection control practices in neonatal units can reduce neonatal mortality and morbidity.

REFERENCES

1. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966;45(4):493-6. erformanceStandardsforAntimicrobial Susceptibility Testing; Twenty-Forth InformationalBukhari EE, Alrabiaah AA. A review of clinically suspected sepsis and meningitis in infants under 90 days old in a tertiary care center in Saudi Arabia. J Microbiol Infect Dis. 2011;1(2).
2. Chelliah A, Thyagarajan R, Katragadda R, Leela KV, Babu RN. Isolation of MRSA, ESBL and AmpC- β-lactamases from neonatal sepsis at a tertiary care hospital. J Clin Diagnostic Res. 2014;8(6):24-7.
3. Collee JG, Marr W. Culture of Bacteria. In: Collee JG, Marmion BP, Simmons A, eds. Mackie and McCartney practical Medical Microbiology. 14th ed. Churchill-Livingstone, New York; 1996:113-129.
4. Gandhi S, Ranjan KP, Ranjan N, Sapre N, Masani M. Research article incidence of neonatal sepsis in tertiary care hospital: an overview. Int J Med. 2013;2(3):548-52.
5. Gerdes JS. Diagnosis and management of bacterial infections in the neonate. Pediatr Clin North Am. 2004;51(4):939-59. Supplement 2014.
6. Jyothi P. Bacteriological profile of neonatal septicemia and antibiotic susceptibility pattern of the isolates. 2013;4(2):2-5.
7. Malik GK. Neonatal sepsis: past, present and future; a review article. Internet J Med Updat. 2010;5(255):45-54.
8. Sankar MJ, Agarwal R, Deorari AK, Paul VK. Sepsis in the new-born. Indian J Pediatr. 2008;75(3):261-6.
9. Sharma CM, Agrawal RP, Sharan H, Kumar B, Sharma D, Bhatia SS. "Neonatal Sepsis": bacteria & their susceptibility pattern towards antibiotics in neonatal intensive care unit. J Clinical Diagnostic Res: JCDR. 2013;7(11):2511-3.
10. Stoll BJ. Infections of the neonatal infant. Nelson Textbook of Pediatrics. Philadelphia, New York: Saunders; 2004:623-640.

Bacteriological Profile and Antibiogram of Blood Culture Isolates from Septicaemic Neonates and Children up to 10 Years of Age, in a Tertiary Care Centre of Eastern Bihar in India

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ABSTRACT

BACKGROUND

This study was an attempt to find the association of physical parameters, risk factors, common signs & symptoms of septicaemia, analyse the distribution of microorganisms isolated from clinically suspected cases of septicaemia, and collect their antibiogram. We also wanted to evaluate the haematological findings in conventional culture, correlate them to the sensitivity and specificity, and quantitatively identify the relevance of these haematological tests through their positive and negative predictive values.

METHODS

A total of 350 blood samples were received from patients with clinically suspected cases of blood stream infections (BSI) at the Department of Microbiology for routine culture & sensitivity and were processed using standard microbiological techniques to determine the percentage distribution of bacterial pathogens causing BSI and their antibiotic susceptibility patterns. Mueller-Hinton agar (MHA) with 4 % NaCl was used to detect methicillin resistance.

RESULTS

Of the 350 septicaemic cases, 58.8 % were from neonatal ICU and 41.2 % were from paediatric wards. Maximum culture positivity (45.3 %) was seen in < 28 days age group. Bacterial growth was seen in 62.0 % preterm babies. Probability of sepsis was more with leukopenia (85.4 %) as compared to leucocytosis (68.9 %); positive C-reactive protein (CRP) findings (63.8 %) were more likely to be associated with sepsis as compared to negative CRP findings (2.1 %). Leukopenia (97.5 %) and leucocytosis (96.3 %) had the highest specificity values.

CONCLUSIONS

Low birth weight (LBW) neonates, preterm birth and Caesarean section deliveries are risk factors that predispose neonates to septicaemia. Meropenem can be used in septaemia, but it should be reserved for critical cases, particularly those with multidrug resistant (MDR) bacteria, rather than on routine basis to prevent inadvertent promotion of bacterial resistance. This study showed that leukopenia and CRP are good indicators of sepsis, when used in combination.

KEYWORDS

Blood Stream Infection, Early Onset Septicemia, Late Onset Septicemia

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BACKGROUND

Risk factors predisposing to neonatal septicemia in developing countries include prolonged rupture of membranes, prematurity, birth asphyxia, length of hospital stay, invasive procedures, delivery location, material used in cutting & dressing cord and maternal infections during pregnancy.[1,2]

The present study was conducted to find out the association of physical parameters & risk factors to clinically suspected septicemia cases; and to see the distribution of microorganisms isolated from cases of sepsis among preadolescent children including newborns, and to collect their antibiogram.

The study also reports the haematological findings in conventional culture and tries to correlate them to the level of accuracy (sensitivity and specificity) with which they were seen to be associated with sepsis.

METHODS

This cross-sectional study was carried out in the Department of Microbiology of a tertiary care centre in Eastern Bihar, in India from May 2019 to November 2020. All patients admitted to the Pediatrics Department of the tertiary centre, with signs of sepsis were included in the study. Clearance was obtained before conducting the study vide IEC / Dept. Res. / 005 / 2019 - 2022 (Microbiology) dated 20.04.2019.

Study Population

Study population was calculated based on a total number of 840 samples received during the study period and calculated on the basis of confidence level of 95 %, and a confidence interval of 4.0. Based on the calculations, a total of 350 blood samples from clinically suspected cases of blood stream infection (BSI) were chosen by simple random sampling and processed for routine blood culture and antibiotic sensitivity, preferably during fever spikes before beginning empirical antibiotic therapy. The study population included all neonates, with age < 28 days to children up to 10 years of age.

Isolation & Identification

The blood culture bottles containing specimens were transported within half an hour to the bacteriology laboratory, incubated at 35^0 C for 7 days and examined macroscopically for appearance of turbidity as evidence of growth during the first 12 - 18 hours after collection. Subcultures were examined for growth on next day. If there was any growth, the isolates were identified as per standard protocol based on standard protocol. If there was no growth, further subculture was done on the 2^{nd}, 4^{th} and the 6^{th} day. Cultures were reported negative, if subcultures showed no growth by then.[3]

Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method on Muller-Hinton agar, using antibiotic discs obtained from HiMedia Laboratories, Mumbai, India. Blood agar was used for *Streptococcus pneumoniae*.[4]

Data Analysis

Performance indices were calculated for haematological parameters like (leukopenia, leukocytosis and CRP). Variables measured were the number of true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN). Sensitivity was calculated as TP / (TP + FN), specificity was calculated as TN / (TN + FP), the PPV was calculated as TP / (TP + FP) and NPV was calculated as TN / (TN + FN).

Statistical Analysis

Statistical analysis of data was done using the online application available at the website link http://www.physics.csbsju.edu/stats/contingency_NROW_NCOLUMN_form.html.

RESULTS

A total of 350 blood cultures were performed during the study period, out of which, 206 (58.8 %) were from neonatal ICU and the remaining 144 (41.2 %) were from the pediatric ward. Majority (145 / 350; 41.4 %) of the children belonged to the age group < 28 days (Table 1). Of the clinically suspected septicaemic cases of neonates, 58.5 % (205 / 350) were males and 41.4 % (145 / 350) were females. The overall male to female ratio was 1: 0.71 (Table 1).

Out of all the culture positive septicaemic cases, maximum culture positivity was seen in low birth weight (LBW) neonates (49.1 %; 53 / 108). Culture positivity in normal birth weight (NBW) neonates was lower (26.8 %; 29 / 108). Maximum bacterial growth was seen in preterm babies (62.0 %; 67 / 108) as compared to the term babies (37.9 %; 41 / 108) (Table 2).

Place of delivery seemed to be the major risk factor and majority of cases (50.9 %; 55 / 108) suffered from sepsis among children whose records showed that they were born in other institutions. It was much lesser when the delivery was in our hospital (31.5 %; 34 / 108), and the least during planned and attended home deliveries (17.5 %; 19 / 108) (Table 2).

Children born of Caesarian sections appeared to be more at risk of BSI (78.7 %; 85 / 108), as compared to those born through normal deliveries (21.3 %; 23 / 108) (Table 2).

The observed values in relation to birth weight ($P < 0.001$), gestational age ($P < 0.001$) and place of delivery ($P < 0.001$) were all statistically significant, while the P-value for the mode of delivery ($P > 0.05$) was statistically insignificant (Table 2).

Of the total 350 blood samples cultured, 184 (52.6 %) were sterile, 108 (30.9 %) showed growth of various bacteria, 20 (5.7 %) were candida species positive and 38 (10.9 %) showed growth of contaminants. Among positive

blood culture isolates, the gram-positive bacterial isolates (47.2 %; 51 / 108) marginally exceeded the gram-negative isolates (45.3 %; 49 / 108).

Isolation of gram-positive cocci (GPC) (64.4 %; 38 / 59) and fungi (8.4 %; 5 / 59) were more (26.5 %; 13 / 49) from late onset septicaemia cases (LOS) and lower (67.3 %; 33 / 49) in early onset septicemia (EOS) (Table 3).

The predominant signs and symptoms were poor activity / poor feeding (53.1 %; 186 / 350), followed by fever (21.7 %; 76 / 350), vomiting (8.2 %; 29 / 350) and convulsions (6.5 %; 23 / 350).

Gram negative bacilli (GNB) showed maximum resistance to cefuroxime, cotrimoxazole & piperacillin-tazobactam, being 76.3 % (29 / 38) each. Most of the GNB were found to be sensitive to colistin (65.7 %; 25 / 38), followed by meropenem (42.1 %; 16 / 38), amikacin (39.4 %; 15 / 38) and netilmicin (34.2 %; 13 / 38); (Table 4).

Pseudomonas aeruginosa showed maximum resistance to cefotaxime (100.0 %; 11 / 11) followed by netilmicin, nalidixic acid & piperacillin-tazobactam (90.9 %; 10 / 11, each) and aztreonam & gentamicin (81.8 %; 9 / 11, each) (Table 4).

GPC showed maximum resistance to aztreonam (68.6 %; 35 / 51), followed by meropenem & cotrimoxazole (64.7 %; 33 / 51, each), and erythromycin (58.8 %; 30 / 51). Resistance to vancomycin was shown by 33.3 % (17 / 51) isolates. Only 39.2 % (20 / 51) isolates showed resistance to cefoxitin (Table 4).

Routine haematological investigation profile was recorded and analysed as shown in (Table 5), which depicts that probability of sepsis is more with leukopenia (85.4 %; 35 / 41), as compared to leucocytosis (68.9 %; 20 / 29). Also, positive C-reactive protein (CRP) finding (63.8 %; 104 / 163) was more likely to be associated with sepsis as compared to negative CRP finding (2.1 %; 4 / 187).

Comparison of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of various haematological parameters showed, CRP had the highest sensitivity (96.3 %) and negative predictive value (NPV) (97.9 %), leucopenia (97.5 %) and leucocytosis (96.3 %) had the highest specificity values (Table 5).

Physical Parameters		Sepsis: Culture Positive N	%	Culture Negative N	%	Total Cases N	%	Chi-Square X^2	'P' Value
Gender	Male	63	58.3	142	58.6	205	58.5	0.365	0.952
	Female	45	41.6	100	41.6	145	41.4		
	Total	108	100	242	100.0	350	100.0		
Age	< 28 days	49	45.3	96	39.6	145	41.4	10.7	0.014
	28 days to 1 yr.	27	25.0	56	23.1	83	23.7		
	1 yr. to 5 yrs.	19	17.5	77	31.8	96	27.4		
	5 yrs. to 10 yrs.	13	12.1	13	5.3	26	7.4		
	Total	**108**	**100.0**	**242**	**100.0**	**350**	**100**		
	Age (Mean + SE)	1.583 + 0.235		1.498 + 0.124		--	--	--	--

Table 1. Association between Physical Parameters and Sepsis

Risk Factors	Parameters	Sepsis: Culture Positive No.	%	Culture Negative No.	%	Total Cases No.	%	Chi-Square X^2	'P' Value
Birth weight	VLBW#	26	24.1	5	2.1	31	8.9	67.5	0.000*
	LBW##	53	49.1	78	32.2	131	37.4		
	Normal	29	26.8	159	65.7	188	53.7		
	Total	108	30.8	242	69.1	350	100.0		
Gestation	Term	41	37.9	202	83.5	244	69.7	73.3	0.000*
	Pre term	67	62.0	40	16.5	107	30.5		
	Total	108	30.8	242	69.1	350	100.0		
Place of delivery	Other institution	55	50.9	74	30.5	129	36.8	25.1	0.000*
	Own institution	34	31.5	146	60.3	180	51.4		
	Home delivery	19	17.5	22	9.1	41	11.7		
	Total	108	30.8	242	69.1	350	100.0		
Mode of delivery	CS###	85	78.7	176	72.7	261	74.5	1.41	0.236
	ND####	23	21.3	66	27.3	89	25.4		
	Total	**108**	**30.8**	**242**	**69.1**	**350**	**100.0**		

Table 2. Association of Clinically Suspected Cases by Risk Factor

#VLBW = Very Low Birth Weight, ## LBW = Low Birth Weight, ###CS = Caesarian Section, ####ND = Normal Delivery, *indicates significant statistical association at P < 0.05

Organism Type	Onset of Septicemia: EOS* N (%)	LOS** N (%)	Total N (%)
Gram positive	13 (26.5)	38 (64.4)	51 (47.2)
Gram negative	33 (67.3)	16 (27.1)	49 (45.3)
Fungi	3 (6.2)	5 (8.4)	8 (7.4)
Total	**49**	**59**	**108**
Chi-square 'P' value	X^2 =17.88, P = < 0.0001*		

Table 3. Organism Type vs. Onset of Septicaemia

*EOS = Early Onset Septicemia, ** LO = Late Onset Septicemia, *indicates significant statistical association at P < 0.05

Antimicrobial	GNB (N = 38) Resistant (%)	GNB (N = 38) Sensitive (%)	*Pseudomonas aeruginosa* (N = 11) Resistant (%)	*Pseudomonas aeruginosa* (N = 11) Sensitive (%)	GPC (N = 51) Resistant (%)	GPC (N = 51) Sensitive (%)
Amikacin	15 (39.4)	15 (39.4)	7 (63.6)	6 (54.5)	11 (21.5)	31 (28.7)
Amoxycillin	26 (68.4)	12 (31.5)	-	-	23 (45.0)	19 (37.3)
Aztreonam	28 (73.6)	10 (26.3)	9 (81.8)	6 (54.5)	35 (68.6)	16 (31.3)
Cefazolin	-	-	-	-	15 (29.4)	31 (60.7)
Cefotaxime	23 (60.5)	7 (18.4)	11 (100.0)	2 (18.2)	-	-
Cefoxitin	-	-	-	-	20 (39.2)	31 (60.7)
Ceftazidime	-		8 (72.7)	5 (45.4)	-	-
Cefuroxime	29 (76.3)	6 (15.7)	-		-	-
Ciprofloxacin	24 (63.1)	9 (23.6)	8 (72.7)	7 (63.6)	29 (56.8)	19 (37.2)
Clindamycin	-	-	-	-	15 (29.4)	23 (45.0)
Colistin	13 (34.2)	25 (65.7)	5 (45.4)	10 (90.9)	-	-
Cotrimoxazole	29 (76.3)	16 (42.1)	5 (45.4)	5 (45.4)	33 (64.7)	12 (23.5)
Erythromycin	-	-	-	-	30 (58.8)	20 (39.2)
Gentamicin	20 (52.6)	8 (21.0)	9 (81.8)	2 (18.2)	16 (31.3)	24 (47.0)
Imipenem	22 (57.8)	11 (28.9)	7 (63.6)	5 (45.4)	-	20 (39.2)
Levofloxacin	20 (52.6)	11 (28.9)	5 (45.4)	10 (90.9)	-	-
Linezolid	-	-	-	-	9 (17.6)	34 (66.6)
Meropenem	19 (50.0)	16 (42.1)	7 (63.6)	8 (72.7)	33 (64.7)	18 (35.3)
Nalidixic acid	28 (73.6)	10 (26.3)	10 (90.9)	5 (45.4)	16 (31.3)	35 (68.6)
Netilmicin	25 (65.7)	13 (34.2)	10 (90.9)	5 (45.4)	25 (49.0)	20 (39.2)
Piperacillin-tazobactam	29 (76.3)	9 (23.6)	10 (90.9)	5 (45.4)	-	-
Vancomycin	-	-	-	-	17 (33.3)	26 (50.9)

Table 4. Antimicrobial Susceptibility Pattern of Organisms Isolated from Septicaemic Cases

Haematological Parameter Variants	Parameter Present (P) / Absent (A)	Conventional Culture Positive (N = 108)	Conventional Culture Negative (N = 242)	Total N = 350	Positivity (%)	Sensitivity	Specificity	PPV	NPV
Abnormal leukocyte count	P	43 (TP)	27 (FP)	70	61.4	74.1 %	88.8 %	61.4 %	93.5 %
	A	65 (FN)	215 (TN)						
Leucopenia (< 5000 / mm^3)	P	35 (TP)	6 (FP)	41	85.4	32.4 %	97.5 %	85.4 %	32.4 %
	A	73 (FN)	236 (TN)						
Leucocytosis (> 20,000 / mm^3)	P	20 (TP)	9 (FP)	29	68.9	18.5 %	96.3 %	68.9 %	72.6 %
	A	88 (FN)	233 (TN)						
Platelet count (< 100000 / dl)	P	49 (TP)	110 (FP)	159	30.8	45.4 %	54.6 %	30.8 %	69.1 %
	A	59 (FN)	132 (TN)						
C-reactive protein (positive, > 6 mg / dl)	P	104 (TP)	59 (FP)	163	63.8	96.3 %	75.6 %	63.8 %	97.9 %
	A	4 (FN)	183 (TN)						
C-reactive protein (negative, < 6 mg / dl)	P	4 (TP)	183 (FP)	187	2.1	3.7 %	24.4 %	2.1 %	36.2 %
	A	104 (FN)	59 (TN)						

Table 5. Evaluation of Haematological Parameters with Conventional Culture Positivity

DISCUSSION

The present study was an attempt to find out the association of physical parameters & risk factors to clinically suspected cases of septicaemia; the common signs & symptoms associated with clinical suspicion of septicaemia; and to analyse distribution of microorganisms isolated from cases of neonatal sepsis and collect their antibiogram. The study also reports the haematological findings in conventional culture and to correlate them to the level of accuracy (sensitivity and specificity) with which they were seen to be associated with sepsis and to quantitatively identify the relevance of these haematological tests through their PPV and NPV.

Out of the total clinically suspected cases of neonates, 58.5 % (205 / 350) were males and 41.4 % (145 / 350) were females. Among the males, culture positivity was 58.3 % (63 / 108) and for females it was 41.6 % (45 / 108). This was comparable to the other studies.[5] The reason for male preponderance is unknown, but could be due to gender-dependent factors.

In the present study, the total number of LBW babies were 37.4 % (131 / 350) clinically suspected neonates, amongst which culture positivity was seen in 49.1 % (53 / 131) cases. This finding was close to the outcome of the study conducted by other workers, where clinical sepsis was more commonly associated with LBW newborns (60 %).[6]

Our study shows that out of the total of 350 blood samples that were cultured, around half the samples (52.6 %; 184 / 350) were sterile. 30.9 % (108 / 350) samples showed growth of various pathogenic bacteria, while 5.7 % (20 / 350) were candida species positive**.** Other studies have reported similar findings earlier, where the growth of pathogenic bacteria was seen in 29.5 % samples, while 63.8 % showed no growth after 7 days of incubation.[7] Contrasting findings of both bacteraemia (88.9 %) and fungemia (11.1 %) in higher percentages of infected individuals have also been reported.[8] Low blood culture isolation rate in the current study might be due to prior treatment through antibiotics before blood collection either to the mother or to the baby ; or the possible infection of neonates with viruses, other fungi or anaerobes, which cannot be ruled out, but were not detected in the present study.[9,10,11]

The present study results showed 9.7 % (34 / 350) growth of contaminants in the cultures. The common contaminants were bacillus species, micrococcus species, and diphtheroids. In contrast, other studies have reported bacillus species and micrococcus species (37.0 % each), *Staphylococcus epidermidis* (14.8 %) and diphtheroids (11.1 %) as contaminants.[12]

Findings of other studies reported predominance of gram-negative isolates like acinetobacter species, enterobacter species and salmonella species In BSI.[13] Among the gram positive pathogens, coagulase-negative staphylococci (CoNS) and *Staphylococcus aureus* were the most common isolates in our study. A higher prevalence rate of septicaemia was recorded by other workers, due to GNB (67.5 %), than due to gram-positive bacteria (32.5 %).

It was found in our study that, while gram positive bacterial pathogens contributed more to LOS (64.4 %), gram negative pathogens contributed more to EOS (67.3 %). This finding was close to findings of some other studies where LOS was most common, with CoNS as the predominant gram-positive pathogen (67.6 %), while gram negative isolates were predominant in EOS.[14]

Regarding the physical status of neonates at the time of presentation, our study showed that slightly more than half the neonates (53.1 %; 186 / 350) presented with poor activity / poor feeding, fever (21.7 %; 76 / 350), vomiting (8.3 %; 29 / 350) and convulsions (6.6 %; 23 / 350). A study in South India reported 72 % cases with poor activity / poor cry, followed by 10.67 % with respiratory distress and 8 % with convulsions in their study.[15]

In our study, gram negative isolates showed maximum sensitivity to colistin (65.7 %), followed by meropenem (42.1 %), amikacin (39.4 %) and netilmicin (34.2 %). On the other hand, *Pseudomonas aeruginosa*, though a gram-negative bacteria, showed maximum sensitivity to levofloxacin and colistin (90.9 %, each), followed by meropenem (72.7 %) and ciprofloxacin (63.6 %). In a different study, all the gram-positive pathogenic isolates were sensitive to linezolid, tigecycline and vancomycin. Isolates were also sensitive to cotrimoxazole (78.8 %), ceftriaxone (77 %), azithromycin (76.9 %), cefepime (60 %), erythromycin (59.6 %) & clindamycin (53.9 %).[16]

Sensitivity to isolates appears to vary greatly from one place to another and is likely to be a result of local antibiotic pressure which, in turn, is influenced by local prescription practices. An Italian study reported varied isolate specific drugs to treat neonatal BSI, wherein *Escherichia coli* was highly sensitive (100 %) to amikacin, klebsiella to ciprofloxacin (100 %), enterobacter to cotrimoxazole (90 %)

& ciprofloxacin (88.9 %) and group B streptococci to ampicillin.

In our study leukopenia was associated with a greater probability of sepsis (85.4 %). This is in concordance with studies which showed a 42 % blood culture positivity rate with leukopenia and only a 29 % culture positivity rate in cases with total leucocyte count (TLC) > 5000 / cu mm.[17]

Abnormal TLC was seen in 61.4 % cases, while thrombocytopenia was seen in 69.1 % cases in the present study. These findings were not concordant with reports of some authors, who showed a higher percentage of abnormal TLC, and much lower percentage of thrombocytopenia.[18] This wide difference in the haematological findings can be attributed to the methodologies while performing the tests.

In our study, leucocytosis had a high specificity (96.3 %) and low sensitivity (18.5 %) which means that it is more accurate in excluding sepsis. Its PPV was high (68.9 %) indicating that the probability of sepsis in subjects with leucocytosis was high. CRP also had the highest sensitivity (96.3 %) amongst all tests, meaning that in comparison to leucocytosis and leukopenia, CRP can identify those with sepsis more accurately.

Our study showed that WBC count < 5000 / cu mm was found to have a specificity of 97.5 %, but sensitivity of 32.4 %. Other findings showed that leucocytosis had an NPV of 72.6 %. CRP had a high NPV (97.9 %) and the lowest PPV (63.8 %). In a different study, a WBC count < 5000 / cu mm was found to have a specificity of 94 % but a sensitivity of only 50 %.[19] In yet another study, sensitivity, specificity, NPV and PPV for leucopoenia were 22 %, 68 %, 29 % and 59 % respectively.[20]

CONCLUSIONS

LBW neonates, preterm birth, and Cesarean section deliveries are risk factors that predispose neonates to septicaemia. Meropenem can be used in septicaemia, but it should best be reserved for critical cases when patient does not respond to other antibiotics particularly in case of MDR bacteria, rather than on routine basis to prevent inadvertent promotion of bacterial resistance. This study showed that leukopenia and CRP are good indicators of sepsis, when used in combination.

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REFERENCES

[1] Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: When? Where? Why? The Lancet 2005;365(9462):891-900.
[2] Abma W, Khalil MI, Shamsuzzaman AKM, et al. Bacteriological profiles of blood culture isolates by BacT/ALERT 3D automated system. Journal of Shaheed Suhrawardy Medical College 2009;1(2):21-26.
[3] Patricia TM. Overview of bacterial identification methods and strategies. In: Bailey & Scott's Diagnostic Microbiology. St. Louis, Missouri: Elsevier 2014: p. 205-247.
[4] Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: 30th edn. Wayne, Pennsylvania. M100–Supplement 23, 2020.
[5] Begum S, Baki MA, Kundu GK, et al. Bacteriological profile of neonatal sepsis in a tertiary hospital in Bangladesh. J Bangladesh Coll Phys Surg 2012;30(2):66-70.
[6] Bhalchandra MH, Ghogare H, Wyawahare A, et al. Clinical and microbiological profile of neonatal sepsis at tertiary care hospital, Aurangabad, Maharastra. National Journal of Medical Sciences 2012;1(2):32-39.
[7] Rahman N, Akhter K, Sen A, et al. Comparison between conventional blood culture and lysis-centrifugation method in suspected cases of sepsis in children in Katihar, Bihar. Journal of Evolution of Medical and Dental Sciences 2019;8(14):1107-1110.
[8] Nidhi P, Ramamurthy S. Microbiological profile and antimicrobial resistant pattern of blood culture isolates, among septicaemia suspected patients. National Journal of Laboratory Medicine 2016;5(1):17-21.
[9] Gohel K, Jojera A, Soni S, et al. Bacteriological profile and drug resistance patterns of blood culture isolates in a Tertiary Care Nephrourology Teaching Institute. Biomedical Research International 2014(1):153747.
[10] Loureiro MM, de Moraes BA, Quadra MRR, et al. Study of multi drug resistant microorganisms isolated from blood cultures of hospitalized newborns in Rio De Janeiro city, Brazil. Brazil Journal of Microbiology 2002;33(1):73-78.
[11] Wasant P, Srisomsap C, Liammongkolkul S, et al. Urea cycle disorders in Thai infants: a report of 5 cases. Journal of Medical Association of Thailand 2002;85(8):720-731.
[12] Henry KN, Mc Limans AC, Wright JA. Microbiological and clinical evaluation of the isolates by lysis-centrifugation blood culture. Journal of Clinical Microbiology 1983;17(15):864-869.
[13] Angyo IA, Opkeh ES, Opajobi SO. Predominant bacterial agents of childhood septicemia in Jos. Nigerian Journal of Medicine 2001;10(2):75-77.
[14] Motara F, Ballot DE, Perovic O. Epidemiology of neonatal sepsis at Johennesburg Hospital. South African Journal of Epidemiology and Infection 2005;20(3):90-93.
[15] Reddy KV, Sailaja K, Ashok A, et al. Clinico-bacteriological profile of neonatal sepsis in rural tertiary care hospital. International Journal of Contemporary Pediatrics 2017;4(4):1259-1262.
[16] Sarangi KK, Pattnaik D, Mishra SN, et al. Bacteriological profile and antibiogram of blood culture isolates done by automated culture and sensitivity method in a neonatal intensive care unit in a tertiary care hospital

in Odisha, India. International Journal of Advances in Medicine 2015;2(4):387-392.
[17] Ahmad I, Ahmed A, Abrar SS, et al. Changing role of neonatal sepsis markers in the era of BacT/Alert: Is there a need to devise a new septic screen? International Journal of Contemporary Pediatrics 2016;3(1):107-112.
[18] Kalpana KL. Clinical profile, outcome of neonatal sepsis and statistical analysis of sepsis screening markers for early diagnosis. Journal of Dental and Medical Sciences 2016;15(11):40-43.
[19] Shrestha S, Singh DS, Shrestha NC, et al. Comparison of clinical and laboratory parameters in culture proven and unproven early onset sepsis in NICU. Kathmandu University Medical Journal 2013;11(44):310-314.
[20] Philip AG, Hewitt JR. Early diagnosis of neonatal sepsis. Pediatrics 1980;65(5):1036-1041.

ORIGINAL ARTICLE

PREVALENCE, IDENTIFICATION AND DISTRIBUTION OF VARIOUS ENTEROCOCCAL SPECIES ISOLATED IN KATIHAR DISTRICT, BIHAR WITH SPECIAL REFERENCE TO VRE.

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ABSTRACT: BACKGROUND: Identification of enterococci to species level in order to determine the species prevalent in this geographic region and also to determine the species-specific antimicrobial susceptibility pattern. **OBJECTIVE:** To detect and determine glycopeptide resistance by screening for vancomycin resistant enterococci (VRE) in both colonized and infected patients. **METHODS:** A total of 123 isolates of enterococcus species were recovered from various clinical and faecal samples of hospitalized patients, from September 2010 to June 2011. Various species of enterococci were identified by standard methods. Vancomycin susceptibility in enterococci was detected by disc diffusion method (DDM), vancomycin screen agar method (VSAM) and agar dilution method to determine minimum inhibitory concentration (MIC). **RESULTS:** E. faecalis was the predominant isolate from the clinical and faecal samples. Multidrug resistance was more in E. faecium than E. faecalis. MIC method could detect 7 VRE and 27 strains with reduced susceptibility to vancomycin. Disk diffusion test and vancomycin screen agar failed to detect 50 % and 29.4% of resistant strains respectively. **CONCLUSION:** Vancomycin resistance was also detected in less virulent strains of enterococcus like E. gallinarum and E. dispar. In laboratories where performance of MIC studies is not feasible, VSAM method should be preferred over the DDM for detection of vancomycin resistance in enterococci.

KEY WORDS: Enterococcus species, vancomycin resistant enterococci (VRE), multi drug resistant enterococci (MDRE)

INTRODUCTION: Although considered benign and opportunistic pathogens, Enterococci have long been known to cause endocarditis in hospitalized patients. Their resistance to several antimicrobial agents, whether intrinsic (low level resistance to penicillin, cephalosporins and aminoglycosides) or acquired ((high level aminoglycoside resistance) is of great concern.[1] This increasing prevalence has been paralleled by the occurrence of vancomycin-resistant strains, which were first reported in 1988.[2] Recently VRE and MDRE have emerged as a leading cause

of nosocomial infections in patients who are either debilitated or as superadded infection especially in surgical and tertiary care units, particularly after organ transplants. These strains are emerging as causes of meningitis and other infections of the central nervous system in neonates and as osteomyelitis, lung infection, urinary tract and pelvic infections in adults. [1]

Antibiotics that achieve high gastrointestinal concentration but are inactive against enterococci favour colonization of the gastro intestinal tract with VRE. Colonization with VRE can lead to serious diseases like urinary tract infections, bacteremia and VRE sepsis which can be fatal. [3]

To the best of our knowledge no study on enterococcus has been carried out in Bihar and Eastern parts of India regarding its characterization to species level and the burden of VRE in this region. The main objectives of the present study were to identify the species of enterococcus isolated from both clinical and faecal samples, which indicated colonization, determine their antimicrobial susceptibility pattern and look for the presence of VRE in the strains isolated.

MATERIAL AND METHODS:

STUDY POPULATION: A total of 123 isolates of enterococcus species were recovered from various clinical and stool samples of hospitalized patients, from September 2010 to June 2011. 60 strains of enterococcus species were isolated from various samples like urine, catheter tip, pus, drainage fluid, tracheal aspirate and blood. An additional 63 strains of enterococcus were also isolated from fecal samples of hospitalized patients who were otherwise not suffering from any other infection, to look for colonization with enterococcus species. The criteria for VRE infection or colonization were: hospitalization for 5 days or more, use of antimicrobials (cephalosporins, aminoglycosides and fluroquinolones).

ISOLATION & IDENTIFICATION: Enterococci were identified using standard methods based on Gram staining, catalase reaction, hydrolysis of bile esculin, growth in 6.5% NaCl, growth at 10ºC and 40ºC, growth at pH 9.6, heat test, hydrolysis of leucine-beta-napthalamide (LAP) and L-pyrrolidonyl-β-napthalamide (PYR). Further identification to species level were based on carbohydrate fermentation using 1% solution of following sugars: glucose, lactose, mannitol, sucrose, arabinose, sorbose, sorbitol, raffinose, ribose, trehalose, xylose, melibiose, glycerol; by pigment production, motility test, pyruvate utilization in 1% pyruvate broth, acidification of methyl-alpha-D-glucopyranoside, Voges-Proskeuer test, arginine decarboxylation, hippurate hydrolysis, reduction of potassium tellurite and tetrazolium chloride.[4, 5, 6]

ANTIMICROBIAL SUSCEPTIBILITY TESTING: Antibiotic susceptibility test was done by Kirby Bauer disc diffusion method on Muller Hinton agar. Inoculum was prepared and adjusted to 0.5 Mc Farland's turbidity standard. Antibiotic disc were obtained from the Hi Media Laboratories (Mumbai) viz ampicillin (10 µgm), ampicillin/sulbactam (10/10 µgm), penicillin (10 units), piperacillin (100µgm), tetracycline (30 µgm), ciprofloxacin (5 µgm), erythromycin (15 µgm), vancomycin (30µgm), teicoplanin (30 µgm), linezolid (30 µgm) & imipenem (30 µgm). The test was quality controlled using E. faecalis ATCC 51299 and E. faecalis ATCC 29212. [7]

Detection of vancomycin resistance in enterococci by vancomycin screen agar method:

For the agar screen method brain heart infusion agar (Hi Media, Mumbai) was supplemented with 6 µgm/ml of vancomycin. The test organisms were grown in peptone water and the turbidity was matched with 0.5 Mc Farland's standard. The bacterial strains were spot inoculated on the agar medium using 10µl of bacterial culture. The plates were incubated at 37ºC for 24 hours. Presence of more than one colony or a haze of growth after 24-hour incubation was read as resistance. [7, 8]

Determination of MIC by agar dilution method: Agar dilution was used to determine MIC of vancomycin to enterococci. Brain-heart infusion agar (Hi Media, Mumbai) was supplemented with different concentrations of vancomycin. The test organism was grown in broth and the turbidity matched with Mc Farland's 0.5 standard. The bacterial strains were spot inoculated on the surface of agar medium using 10 µl of bacterial culture. The plates were incubated at 37ºC for 24 hours. The minimum concentration of vancomycin, which inhibited bacterial growth, was considered MIC. Enterococci which had MIC ≥ 32µgm/ml were considered resistant; MIC of 8-16 µgm/ml as intermediately resistant; and MIC of ≤ 4 µgm/ml as susceptible to vancomycin. [8]

RESULTS: Out of the 60 enterococcal strains isolated from clinical samples, 27(46%) were identified as E. faecalis, 26 (43%) as E. faecium, 3 (5%) as E. solitarius and 2 (3.3%) each as E. raffinosus and E. gallinarum. The majority of the isolates from the fecal samples were E. faecalis 28 (44.4%) followed by E. gallinarum 22 (34.9%), E. faecium 11 (17.7%) and 1(1.5%) each of E. raffinosus and E. dispar.

Enterococcal strains, both clinical isolates and isolates from faeces of colonized patients showed resistance to various antibiotics. Antibiotics to which maximum resistance was seen were piperacillin, ciprofloxacin, penicillin, ampicillin and imipenem. Clinical isolates showed least resistance to vancomycin, teicoplanin and linezolid whereas faecal isolates showed least resistance to linezolid, teicoplanin and vancomycin. However vancomycin resistance was seen in 14.2% of faecal isolates as compared to 1.6% of clinical isolates.(**Table 1**) Amongst the faecal isolates, 27.2% of E. faecium were found to be VRE and 10.7% of E. faecalis. Multidrug resistance amongst clinical isolates was more in E. faecium; 73% of these strains showing complete resistance to imipenem.(**Table 1**) Out of the total of 123 clinical and faecal isolates, 17 and 24 isolates were resistant to vancomycin by disc diffusion method(DDM) & vancomycin agar screen method(VASM).(**Table2**) The highest resistance was observed among E.faecalis followed by E. faecium and E. gallinarum by agar screen method.

MIC test of various clinical strains of enterococcus to vancomycin showed 8 strains to have reduced susceptibility to vancomycin i.e. MIC ranging from 8 to 16 µgm/ml. Only one strain of E. faecium had MIC ≥ 32µgm/ml and was considered as VRE. **(Table 3)**Faecal isolates on the other hand were found to be more resistant to vancomycin with 19 strains showing reduced susceptibility and six showing resistance i.e. MIC ≥ 32µgm/ml.**(Table 4)**

Amongst the 9 clinical VRE and or VIE, 4 (44.4%) strains were isolated from urinary tract infections (UTI), 3 (33.3%) from wound infection and 2 (22.2%) from blood stream infection (BSI). On the other hand 25 faecal isolates were found to be VRE/VIE. The predominant clinical presentations in the VRE positive cases were appendicitis, hernia, burn, granuloma, swelling of body and hepatosplenomegaly.

DISCUSSION: Combination of colonizing abilities and drug resistance both inherent and acquired, has made Enterococci attain greater significance as human pathogens. In the present

study, E. faecalis was the predominant species isolated from both clinical & fecal specimens. Other studies done on Enterococci also support the same finding. This is probably due to predominance of E. faecalis in the endogenous flora of human body. [9, 10]

The major sources of VRE are from the clinical environment. [11, 12] In our study, VRE strains were isolated from faecal samples of colonized patients. The VRE species isolated from these samples were E. faecium followed by E. faecalis, E. gallinarum and E. dispar. Patients infected or colonized with VRE did not show any clear correlation between the use of antimicrobials or gastrointestinal tract surgery.

The incidence of infection with strains of enterococcus with glycopeptides resistance has increased dramatically. In the present study DDM and VASM test failed to detect 50% and 29.4% strains respectively. The DDM failed to recognize as resistant those strains that have reduced susceptibility to vancomycin. The MIC test not only detected 7 VRE but also another 27 enterococcal strains with reduced susceptibility to vancomycin. The MIC test may therefore be considered as the gold standard. This observation is consistent with that made in a previous report. [8]The treatment of vancomycin resistant enterococci is a major problem. Vancomycin resistance eliminates the synergistic activity usually achieved by aminoglycoside combination, thus leaving beta-lactams as the only choice to combine with aminoglycoside. Moreover many of the vancomycin resistant enterococci are multidrug resistant. The antibiotic of choice for such multidrug resistant enterococci is currently not known. [8]

Out of the 63 stool isolates, 9 strains were resistant to 9 antibiotics out of the 10 antibiotics used. The outcome of infections with such multidrug resistant Enterococcus strains might be fatal. In most of the earlier reports, such high MDR rates were rarely observed in enterococci. High resistance of the clinical strains of E. faecalis to penicillin could be due to low affinity of penicillin binding proteins or production of beta lactamases. [9]

We conclude that enterococcal strains with high rate of resistance to multiple drugs are not only prevalent in the clinical environment but also in the gastrointestinal tract of the colonized patients. However patients with VRE infections and those showing resistance to multiple drugs could not be followed up, so the actual outcome of the infections with these strains could not be found out.

The prevalence of VRE was quite high amongst the colonized patients. This situation makes it mandatory for the clinical microbiologists to try to identify the most useful active antibiotic for treatment. The time has come for proper control measures to be taken to prevent the spread of such infections. MIC for vancomycin should be performed, in laboratories equipped to perform these tests to keep record of increasing resistance of enterococci to vancomycin.

REFERENCES:

1. Singh BR. Prevalence of vancomycin resistance and multiple drug resistance in enterococci in equids in North India. J Infect Dev Ctries 2009; 7:498-503.
2. Schouten MA, Hoogkamp-Korstanje JAA, Meis JFG, Voss A. Prevalence of Vancomycin-Resistant Enterococci in Europe. J Clin Microbiol Infect Dis 2000; 19:816-866
3. Javadi A, Ataei B, Khorvash F, Toghyani S, Mobasherzadeh S, Soghrati M. Prevalence of Vancomycin resistant enterococci colonization in gastrointestinal tract of hospitalized patients. Iranian J of Clin Infect Dis 2008;3: 137-141

4. Desai PJ, Pandit D, Mathur M, Gogate A. Prevalence, identification and distribution of various species of enterococci isolated from clinical specimens with special reference to urinary tract infection in catheterized patients. Indian J Med Microbiol 2001; 3:132-137
5. Facklam R, Collin. Identification of enterococcus species isolated from human infection by a conventional test scheme. J Clin Microbiol1989; 27:731-734
6. Overview of Bacterial Identifications Methods and Strategies. In: Bailey & Scott's Diagnostic Microbiology.12th ed. Forbes Betty A, Sahm Daniel L, Weissfeld Allice S, editors. (Mosby Inc, Missouri; 2007: 216-241)
7. Swenson Jana M, Patel Jean B, Jorgensen James H. Special Phenotypic Methods for Detecting Antibacterial Resistance. Vol. 1.9th ed. Murray Patrick R, Baron Ellen Jo, Jorgensen James H, Landry Marie Louise and Pfaller Michael A, editors. (ASM Press, Washington D.C, 2007: 1152-1172.)
8. Adhikary L. High-level aminoglycoside resistance and reduced susceptibility to vancomycin in nosocomial enterococci. J. of Global Infectious Disease 2010; 2:231-234.
9. Gupta V, Sigla N. Speciation and antimicrobial susceptibility pattern of enterococci from a tertiary health care center of North India. J of Clin and Diag Res 2007; 1: 385-389.
10. Marcus N. Rapid increase in the prevalence of antimicrobial drug resistance among enterococcal blood isolates in southern Isreal. Eur J Clin Microbiol Infect Dis 1997; 45: 79-82
11. Woodford N. Glycopeptide – resistant enterococci: a decade of experience. J Med Microbial 1998; 47: 849-862.
12. Ooi WL, Haryiani Y, Lesley M.B., Tunung R., Jurin G., Wong CMVL. Vancomycin Resistant Enterococci (VRE) In Poultry, Vegetables, Enviornmental and Clinical Sources. Malays Appl Biol 2006; 35: 49-56.

Table 1: Resistance pattern of different enterococcus species

Antibiotics	Clinical Isolates (n=60) No. of resistant strains					Fecal Isolates (n=63) No. of resistant strains				
	E.faecalis	*E.faecium*	*E.solitarius*	*E.raffinosus*	*E.gallinarum*	*E.faecalis*	*E.faecium*	*E.gallinarum*	*E.dispar*	*E.raffinosus*
Penicillin	22 (81.4)	12 (46.1)	0	0	0	20 (71.4)	3 (27.2)	0	0	0
Ampicillin	7 (25.9)	12 (46.1)	0	0	0	13 (46.4)	4 (54.5)	9 (40.9)	1 (100)	0
Ampicillin/sulbactam	10 (37)	10 (38.4)	0	0	0	16 (57.1)	7 (63.6)	11 (50)	1 (100)	0
Tetracycline	10 (37)	10 (38.4)	1 (33.3)	1 (50)	1 (50)	20 (71.4)	6 (54.5)	7 (31.8)	0	0
Piperacillin	25 (92.5)	20 (76.9)	1 (33.3)	1(50)	1(50)	28 (100)	10 (90.9)	19 (86.3)	1 (100)	1 (100)
Ciprofloxacin	24 (88.8)	12 (46.1)	2 (66.6)	1 (50)	0	27 (96.4)	9 (81.8)	15 (68.1)	0	1 (100)
Linezolid	2 (7.4)	3 (11.5)	0	0	0	1 (3.5)	2 (18.1)	1(4.54)	0	0
Imipenem	10 (37)	19 (73)	0	0	0	20 (71.4)	7 (63.6)	19 (86.3)	0	0
Vancomycin	0	1 (3.84)	0	0	0	3 (10.7)	3 (27.2)	2 (9.1)	1 (100)	0
Teicoplanin	1 (3.7)	0	0	0	0	3 (10.7)	2 (18.1)	0	0	0

Figures in parenthesis indicate percentages

Table 2: Distribution of resistant strains of enterococcus species detected by both DDM & VSAM:

Methods used to detect vancomycin resistance	Clinical Isolates (n=60) No. of resistant strains					Faecal Isolates (n=63) No. of resistant strains				
	E.faecalin (27)	*E.faecium* n (26)	*E.solitarius* n (3)	*E.raffinosus* n (2)	*E.gallinarum* n (2)	*E.faecalis* n (28)	*E.faecium* n (11)	*E.gallinarm* n (22)	*E.dispar* n (1)	*E.raffinosus* n (1)
Disc-diffusion method (strains showing intermediate-resistance & completee resistant	2	3	0	0	0	5	4	2	0	1
TOTAL	5					12				
Vancomycin gar screen method	3	4	0	0	0	7	5	4	0	1
TOTAL	7					17				

Table-3: MIC range for vancomycin (µgm/ml) in different clinical isolates

Clinical isolates	MIC Values (µgm /mm)						
	≤ 0.5	1	2	4	8	16	≥32
E. faecalis (N=27)	0	0	16	9	3	0	0
E. faecium (N=26)	0	2	16	5	4	0	1
E. raffinosus (N= 2)	0	0	2	0	0	0	0
E. solitarius (N= 3)	0	0	3	0	0	0	0
E. gallinarum (N= 2)	0	0	1	0	1	0	0

Table-4: MIC range for vancomycin (µgm/ml) in different faecal isolates

Stool Isolates	MIC Values (µgm /mm)						
	≤ 0.5	1	2	4	8	16	≥ 32
E. faecalis (N=28)	0	1	9	9	2	6	1
E. faecium (N=11)	0	1	4	3	2	4	2
E. gallinarum (N=22)	0	7	7	0	0	4	2
E. raffinosus (N=1)	0	0	1	0	0	0	0
E. dispar (N=1)	0	0	0	0	1	0	1

ORIGINAL ARTICLE

Detection of vancomycin resistance in enterococcus species isolated from clinical samples and feces of colonized patients by phenotypic and genotypic methods

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ABSTRACT

Background: The aim of this study was to find out the clinical correlation between the presence of vancomycin-resistant genes (*van A* and *van B*) and their expression as detected by phenotypic tests in colonized patients and in clinical isolates. **Materials and Methods:** *Enterococci* were isolated from various clinical samples and also from fecal specimens of colonized patients at the time of admission, after 48 h and after 5 days of admission. Identification to species level was done using standard methods. Vancomycin susceptibility in *Enterococci* was detected by disc diffusion test. Minimum inhibitory concentration was determined by agar dilution method. Multiplex polymerase chain reaction (PCR) was used to detect the presence of *van* genes. **Results:** Out of all the clinical and fecal samples processed, 12.0% isolates were either vancomycin resistant or vancomycin intermediate. Further, these isolates carried *van A* or *van B* genes as confirmed by PCR methods. Expression of *van A* gene was found to be more in *Enterococcus faecalis* (28.3%) as compared to *Enterococcus faecium* (25.0%) in both clinical and fecal isolates. 16.6% strains of *E. faecium* and 15.0% strains each of *E. faecalis* and *Enterococcus gallinarum* were found to carry *van B* genes. The overall prevalence of vancomycin resistant *Enterococci* (VRE) in colonized patients was about 9.6%. Prior administration of antibiotics had significant effect ($P = 0.001$) on VRE carriage. Urinary tract infection was the most common infection caused by vancomycin susceptible *Enterococci* (VSE), 105/214 (49.0%) and VRE, 13/36 (36.1%). There was no significant difference ($P = 0.112$) in the distribution of VRE and VSE in different infection types. Both clinical and fecal VRE showed maximum resistance to penicillin, ampicillin, and piperacillin. Resistance to linezolid was 2.8% in clinically isolated VRE. **Conclusion:** VRE in our study were found to be resistant to a number of commonly used antibiotics. The frequency of isolation of vancomycin resistant *E. faecalis* (VR*E.fs*), which is highly virulent, and the number of strains harboring *van A* gene in our hospital setup is high and needs to be addressed.

KEY WORDS: *Van A, van B*, vancomycin resistant *Enterococci*

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INTRODUCTION

Vancomycin resistant *Enterococci* (VRE) have caused hospital outbreaks worldwide, which has been dramatically amplified in recent years, because of widespread abuse and misuse of antibiotics, leading to increase in infections caused by these strains.[1]

Promotion of vancomycin-resistant enterococcal colonization is due to rampant use of vancomycin, and also third generation cephalosporins, imipenem, metronidazole, and clindamycin with potent activity against anaerobes, which lead to VRE colonization of gastrointestinal tract (GIT) by competitive eradication of sensitive species. VRE colonization leads to cross-infection, dissemination, and endogenous infection. Hence, it has been proposed by many workers that attempts must be made to detect the rate of VRE isolation in diseased as well as colonized patients. Therefore, screening for the presence of VRE, in diseased persons only does not provide the total picture of this problem.[2]

The errors associated with the phenotypic disc diffusion method leads to unwarranted use or elimination of this drug from a treatment regimen. Use of multiplex polymerase chain

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reaction (PCR) limits the errors arising out of phenotypic methods.

It is not always easy to assess the clinical significance of VRE isolation in routine culture or to differentiate colonization from infection. Therefore, the present study was undertaken to look for vancomycin resistance in *Enterococci* obtained in significant numbers from various clinical samples as well as fecal samples of colonized patients. The isolation of VRE is found to vary in different geographical regions, and this study was undertaken to determine the vancomycin resistant genotypes prevalent in Eastern Bihar, India.

MATERIALS AND METHODS

Study population

The study population included patients of both sexes and all age groups attending the outpatient and inpatient departments of a tertiary care hospital in Eastern Bihar, India. A total of 500 strains of *Enterococci* were collected from samples submitted to the Microbiology Laboratory for culture and sensitivity. 250 enterococcal strains were collected from clinical samples of the patients, attending the hospital with infections of different types. The second group included another 300 patients (otherwise not suffering from any infections), who were admitted to the hospital and screened for gastrointestinal carriage of VRE. Another 250 enterococcal strains were isolated from this group. Clearance from Institutional Ethics Committee was obtained to carry out this study.

Isolation and identification

Two-hundred and fifty *Enterococci* were isolated from various clinical samples (urine, pus, blood, catheter tip, and tracheal aspirate). 300 fecal samples were collected from other patients, (as mentioned above) on three occasions, that is, at the time of admission, after 48 h, and after 5 days of admission to screen for VRE. The isolates were identified to species level using standard procedures.[3-7]

Antimicrobial susceptibility testing

Antibiotic susceptibility test was done by Kirby-Bauer disc diffusion method on Muller-Hinton agar. Inoculum was prepared and adjusted to 0.5 McFarland's turbidity standard. Antibiotic disc was obtained from the Hi Media Laboratories (Mumbai).[8]

Determination of minimum inhibitory concentration by agar dilution method

Agar dilution was used to determine minimum inhibitory concentration (MIC) of vancomycin. Brain-heart infusion agar (Hi Media, Mumbai) was supplemented with different concentrations of vancomycin. The test organism was grown in broth and the turbidity matched with McFarland's 0.5 standard. The bacterial strains were spot inoculated on the surface of agar medium using 10 µL bacterial culture. The plates were incubated at 37°C for 24 h. The minimum concentration of vancomycin, which inhibited bacterial growth, was considered MIC. *Enterococci* which had MIC ≥32 µg/mL were considered resistant; MIC of 8–16 µg/mL as intermediately resistant; and MIC of ≤4 µg/mL as susceptible to vancomycin.[8,9]

DNA extraction method

Genomic DNA used as template for PCR amplification was prepared using conventional phenol-chloroform DNA extraction method.[10]

The following oligonucleotide primers were used for amplification of 1030 bp of the *van A* gene: F14-CATGAATAGAATAAAAGTTGCAATA and R14-CCCCTTTAACGCTAATACGATCAA and 433-bp of the *van B* gene: F15-GTGACAAACCGGAGGCGAGGA and R15-CCGCCATCCTCCTGCAAAAAA. Primers were obtained from Merck's, Lucknow, India. The total volume of PCR mix was 25 µl including: 2 µl dNTPs mix, 0.3 µl 5U Taq polymerase, 2.5 µl 10X buffer, 4 µl *van A* and 4 µl *van B* primer, and 12.17 µl double distilled water.

Polymerase chain reaction assay for *van A* and *van B* genes

The PCR amplification of the van genes were carried out as per standard protocol.[10] Both positive control (positive VRE culture with known inherited vancomycin positive genes) and negative control, consisting solely of the PCR reaction mixture without DNA template, were included to check the validity of the technique. The amplified products were electrophoresed using 1.5% agarose gel. A 100 bp DNA ladder marker was included as the standard molecular weight marker. The electrophoresed gel was later subjected to ethidium bromide staining and photographed under ultraviolet transillumination[10] [Figure 1]. PCR was performed in PCR system, model number T1 Thermoblock.

Statistical analysis

Statistical analysis was done using Chi-square test. $P < 0.05$ was considered significant and $P < 0.001$ was considered highly significant.

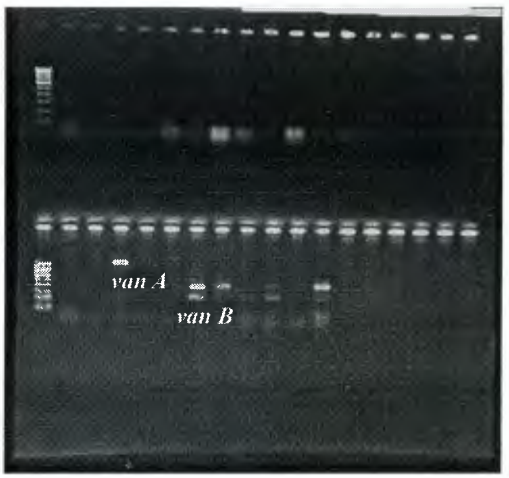

Figure 1: Bands corresponding to 1030 bp (*van A*) and 433 bp (*van B*) in polymerase chain reaction assay. Polymerase chain reaction was performed in polymerase chain reaction system, model number T1 Thermoblock

RESULTS

A total of 500 *Enterococci* (250 from clinical samples and 250 from fecal samples of colonized patients) were processed, out of which 37 (7.4%) isolates were vancomycin resistant and 23 (4.6%) showed reduced susceptibility to vancomycin by phenotypic agar dilution method. However, by disc diffusion test (DDT), 34 (6.8%) strains were found to be resistant to vancomycin and 20 (4.0%) strains were found to show reduced susceptibility.

MIC of various clinical strains of enterococcus to vancomycin showed 11 strains to have reduced susceptibility to vancomycin, that is, MIC ranging from 8 to 16 μg/ml. Another 11 strains of *E. faecalis*, 9 strains of *E. faecium*, and 5 strains of *E. gallinarum* had MIC ≥64 μg/ml, and were considered as VRE. On the other hand, 12 fecal isolates showed reduced susceptibility and another 12 strains (6 strains each of *E. faecalis* and *E. faecium* showed resistance, that is, MIC ≥64 μg/ml [Table 1].

Thus, a total of 60 strains (36 clinical and 24 fecal) which were VRE/vancomycin intermediate *Enterococci* (VIE) by MIC tests were also confirmed to carry *van A* or *van B* genes by PCR methods. 17 strains of *E. faecalis* and 15 strains of *E. faecium* were found to carry *van A gene* and 10 strains of *E. faecium* and 9 strains each of *E. faecalis* and *E. gallinarum* were found to carry *van B* genes. The prevalence of *van A* gene was found to be significantly higher ($P = 0.027$) in the clinical strains [Table 2].

Out of 300 fecal samples collected, 50 (16.7%) samples were culture negative for enterococcus and 250 (83.3%) samples showed growth of enterococcus species. 70 strains each were isolated from gynecology, medicine, and surgery department and 20 strains each from Medical Intensive Care Unit (MICU) and pediatrics departments, respectively. 12 (50.0%), 7 (29.2%), and 1 (4.1%) VRE carriers were from surgery, medicine, and gynecology departments, respectively. On the other hand, 4 (16.6%) VRE carriers were from patients admitted in MICU. The VRE carriage rate was maximum in surgery followed by medicine department.

Out of a total of 250 fecal isolates, VRE were detected in 24 (9.6%) patients, out of which 16 (6.4%) were males and 8 (3.2%) were females. The male to female ratio of patients colonized with VRE was 2:1. The difference in the colonization rate of VRE in both sexes was insignificant ($P = 0.09$) [Table 3]. VRE carriage was maximum in middle age group 41–50 years, 11 (45.8%) followed by 10 (41.7%) in 51–60 years, and 3 (12.5%) in 31–40 years of the 24 VRE patients, rate of colonization was 87.5% (21/24) after 5 days as compared to 12.5% (3/24) after 48 h.

Eighteen out of the 24 patients with VRE colonization had received various antibiotics viz., 41.7% received cephalosporins, 12.5% each received fluroquinolones and metronidazole, and 8.3% received gentamicin. Prior administration of antibiotics had significant effect ($P = 0.001$) on VRE carriage [Table 4]. None of the patients with VRE colonization had preceding Gram-positive bacteremia or had undergone GIT surgery or received vancomycin.

VRE from clinical samples were isolated from patients in the age group 51–60 years, 9 (25.0%); 31–40 years, 8 (22.2%); 41–50 years, and 7 (19.4%); 6 (16.6%) each in 61–70 years and 21–30 years.

Table 1: MIC range for vancomycin (μg/mL) in different clinical isolates

	MIC values (μg/mL)								
	≤0.125	0.5	1	2	4	8	16	32	≥64
Clinical isolates									
Enterococcus faecalis (*n*=64)	0	8	15	20	7	0	3	0	11
Enterococcus faecium (*n*=60)	0	11	16	6	10	3	5	0	9
Enterococcus mundtii (*n*=33)	0	4	7	10	12	0	0	0	0
Enterococcus raffinosus (*n*=28)	0	4	14	4	6	0	0	0	0
Enterococcus solitarius (*n*=9)	0	1	2	3	3	0	0	0	0
Enterococcus malodoratus (*n*=20)	0	3	4	7	6	0	0	0	0
Enterococcus gallinarum (*n*=19)	0	3	3	6	2	0	0	0	5
Enterococcus durans (*n*=17)	0	2	3	7	5	0	0	0	0
Total=250	0	36	64	63	51	3	8	0	25
Feacal isolates									
Enterococcus faecalis (*n*=82)	0	9	20	20	21	1	5	0	6
Enterococcus faecium (*n*=58)	0	6	15	10	19	0	2	0	6
Enterococcus gallinarum (*n*=76)	0	23	15	19	15	0	4	0	0
Enterococcus raffinosus (*n*=7)	0	0	1	2	4	0	0	0	0
Enterococcus hirae (*n*=17)	0	9	0	3	5	0	0	0	0
Enterococcus dispar (*n*=10)	0	3	2	1	4	0	0	0	0
Total=250	0	50	53	55	68	1	11	0	12

MIC: Minimum inhibitory concentration

Table 2: Species specific distribution of *van A* and *van B* genes

Total number of VRE (*n*=60)	Clinical VRE	
	*van A**	*van B*
Enterococcus faecalis (*n*=14)	11	3
Enterococcus faecium (*n*=17)	9	8
Enterococcus gallinarum (*n*=5)	0	5
Total=36	20	16
	Fecal VRE	
Enterococcus faecalis (*n*=12)	6	6
Enterococcus faecium (*n*=8)	6	2
Enterococcus gallinarum (*n*=4)	0	4
Total=24	12	12

*P=0.027. VRE: Vancomycin resistant *Enterococci*

Table 3: Age and sex-wise distribution of the patients harboring VRE in the GIT

Age group	Number of VRE isolated		
	Male (%)*	Female (%)*	Total
1-10 (*n*=20)	0	0	0
11-20 (*n*=8)	0	0	0
21-30 (*n*=55)	0	0	0
31-40 (*n*=72)	2 (12.5)	1 (12.5)	3 (12.5)
41-50 (*n*=54)	7 (43.8)	4 (50.0)	11 (45.8)
51-60 (*n*=32)	7 (43.8)	3 (37.5)	10 (41.7)
61-70 (*n*=9)	0	0	0
Total=250	16 (100)	8 (100)	24 (100)

*P=0.09. VRE: Vancomycin resistant *Enterococci*; GIT: Gastrointestinal tract

Out of the 36 clinical VRE, 13 strains were from the cases of urinary tract infection (UTI) among which the predominant isolate was *E. faecium* 7 (53.8%); 10 strains were from wound infection of which *E. gallinarum* 4 (40.0%) was the major isolate. 5 out of the 9 VRE that caused blood stream infection (BSI) were *E. faecium* (55.5%). Another 4 (50.0%) strains were found to cause catheter induced infection (CII), of which two each were *E. faecalis* and *E. faecium* [Table 5]. Out of the 214 vancomycin sensitive isolates, 105 isolates were from UTI, 69 from wound infection, 25 from BSI, and 15 from catheter related infection. Most of the nonfaecalis, nonfaecium strains were isolated from urine followed by wound infection [Table 5]. No significant difference ($P = 0.112$) in the distribution of VRE and vancomycin susceptible *Enterococci* (VSE) in different infection types was seen.

Table 6 shows the antibiotic resistance pattern of VSE and VRE (clinical) by disk diffusion test. For VSE, maximum resistance was seen with penicillin 84.1%, followed by ampicillin 70.0%, and piperacillin 67.8%. For VRE, maximum resistance was seen with the same antibiotics viz.: penicillin 100%, ampicillin 91.7%, and piperacillin 75.0%. Least resistance was seen with linezolid for both VSE and VRE being 0% and 2.8%, respectively. For fecal VSE, maximum resistance was seen with penicillin 88.5%, followed by piperacillin 66.4%, and ampicillin 64.2%. All strains were sensitive to linezolid. VRE also showed maximum resistance to these 3 antibiotics being 100% for penicillin, 87.5% for ampicillin, and 79.2% for piperacillin. None of the strains was resistant to linezolid.

DISCUSSION

VRE has become an important nosocomial pathogen because of its rapid spread, high mortality rates associated with infections, limited option for treatment, and the possibility of transferring vancomycin resistance genes to other more virulent and more prevalent pathogens such as *Staphylococcus aureus*. We investigated the prevalence of vancomycin resistance in both clinical and fecal isolates (250 each) by phenotypic and genotypic methods. Our study showed correlation between the presence of *van* genes and their expression as detected by MIC tests by agar dilution method. However, the DDT failed to detect 6 strains of VRE/VIE. Overall, 12.0% of the 500 strains isolated were found to be VRE/VIE. Other authors reported the VRE positivity rate to be low as 6.9%.[11] This differences in the prevalence of VRE in different regions are governed by various factors including the use of glycopeptides in humans and animals (growth promoters).

In the present study, 54 (10.8%) isolates were vancomycin intermediate or resistant by DDT. However, by agar dilution method 23 (4.6%), isolates showed reduced susceptibility to vancomycin, that is, MIC ranging from 8 to 16 μg/ml and another 37 (7.4%) isolates showed resistance, that is, MIC ≥ 64 μg/ml, that is, total 60 (12.0%). This observation clearly indicates that DDT may fail to recognize as resistant those enterococcal strains that have reduced susceptibility to vancomycin. Similar observation

Table 4: Administration of antibiotics in patients colonized with VRE and VSE

Antibiotics	*Patients colonized with VRE (n=24)* (%)*	*Patients fram whom VSE isolates were obtained (n=226) (%)*
Patients who received no antibiotic therapy	6 (25.0)	137 (60.6)
Cephalosporins (1st, 2nd and 3rd generation)	10 (41.7)	40 (17.7)
Aminoglycosides (gentamicin)	2 (8.3)	19 (8.4)
Fluroquinolones	3 (12.5)	18 (8.0)
Metronidazole/clindamycin	3 (12.5)	12 (5.3)
Vancomycin	None	None

**P*=0.001. VRE: Vancomycin resistant *Enterococci*; VSE: Vancomycin susceptible *Enterococci*

Table 5: Distribution of VSE and VRE (clinical) in different infection types

	Infection type (%)				*Total (%)*
	UTI	*Waund infection*	*BSI*	*Catheter induced infection*	
VSE					
Enteracoccus faecalis	26 (24.8)	11 (16.0)	9 (36.0)	4 (26.7)	50 (23.4)
Enterococcus faecium	18 (17.1)	12 (17.4)	9 (36.0)	4 (26.7)	43 (20.1)
Enterococcus mundtii	19 (18.1)	12 (17.4)	1 (4.0)	1 (6.7)	33 (15.4)
Enterococcus raffinosus	10 (9.5)	9 (13.0)	5 (20.0)	4 (26.7)	28 (13.1)
Enterococcus solitarius	5 (4.8)	4 (5.8)	0	0	9 (4.2)
Enterococcus malodaratus	11 (10.5)	8 (11.6)	1 (4.0)	0	20 (9.3)
Enterococcus durans	13 (12.4)	3 (4.3)	0	2 (13.3)	18 (8.4)
Enterococcus gallinarum	3 (2.9)	10 (14.5)	0	0	13 (6.1)
Total*	105	69	25	15	214
VRE					
Enterococcus faecalis	5 (38.5)	3 (30.0)	4 (44.4)	2 (50.0)	14 (38.9)
Enterococcus faecium	7 (53.8)	3 (30.0)	5 (55.5)	2 (50.0)	17 (47.2)
Enterococcus gallinarum	1 (7.7)	4 (40.0)	0	0	5 (13.9)
Total*	13	10	9	4	36

**P*=0.112. VRE: Vancomycin resistant *Enterococci*; VSE: Vancomycin susceptible *Enterococci*; UTI: Urinary tract infection; BSI: Blood stream infection

Table 6: Antibiotic resistance pattern of VSE and VRE (clinical and fecal) by DDT

Antibiatics	*Clinical VSE (n=214) (%)*	*Clinical VRE (n=36) (%)*
Ampicillin	150 (70.0)	33 (91.7)
Penicillin	180 (84.1)	36 (100)
Piperacillin	145 (67.8)	27 (75.0)
Tetracycline	130 (60.7)	24 (66.7)
Erythromycin	122 (57.0)	22 (61.1)
Ciprofloxacin	97 (45.3)	19 (52.8)
Imipenem	44 (20.6)	13 (36.1)
Linezolid	0	1 (2.8)
	Fecal VSE (n=226) (%)	*Fecal VRE (n=24) (%)*
Ampicillin	145 (64.2)	21 (87.5)
Penicillin	200 (88.5)	24 (100)
Piperacillin	150 (66.4)	19 (79.2)
Tetracycline	143 (63.3)	18 (75.0)
Erythromycin	140 (61.9)	15 (62.5)
Ciprofloxacin	99 (43.8)	11 (45.8)
Imipenem	50 (22.1)	7 (29.2)
Linezolid	0	0

VRE: Vancomycin resistant *Enterococci*; VSE: Vancomycin susceptible *Enterococci*; DDT: Disc diffusion test

was made in another study, where all the enterococcal strains were susceptible to vancomycin by DDT but showed intermediate resistance to vancomycin (MIC, 8 μg/ml).[12]

Many species of *Enterococci* may be vancomycin resistant but majority are *E. faecium.* In this study, multiplex PCR proved helpful in detecting the *van* genotypes present in this geographic region. In this study, expression of *van A* gene was seen with *E. faecalis* (28.3%) isolates followed by *Enterococus faecium* (25.0%) in both clinical and fecal isolates. Few *E. gallinarum* (15.0%) strains were found to carry *van B* gene. In clinical isolates, prevalence of *van A* was more than *van B,* a finding that was found to be statistically significant ($P = 0.027$). PCR test result of other studies showed that 13 strains of *E. faecium* carried *van A* gene and 1 strain of *E. gallinarum* carried *van C1* gene.[13] This finding is quite different from the findings of this study in which *E. faecalis,* which is a more virulent strain, was predominantly found to carry *van A* gene. As compared with *van B,* the *van A* gene is known to have increased transferability, which may explain the rapid increase in the number of *van A* isolates. Strains of *E. gallinarum* carrying *van B* gene also has important role in the dissemination of antibiotic resistant genes as compared to *E. gallinarum* carrying *van C* genes which is nontransferable and an inherent characteristic.

Majority of the VRE isolated from the colonized patients were from surgery department 50.0% (12/24) followed by medicine 29.2% (7/24), MICU 16.6% (4/20), and gynecology 1.4% (1/70) departments. No VRE was isolated from pediatrics department. Other authors have reported that 30.5% (39/128) patients were colonized with VRE strains: 7.8% (10/39) in the ICU, 10.9% (14/39) in surgery, and 11.7% (15/39) in medicine wards.[14] No VRE strain was isolated from pediatric ward, a finding similar to our study. The authors explain this finding may be due to physical isolation, intrinsic differences in bowel mileu, and lack of exposure to food or other environmental sources.

Majority of the colonized patients with VRE infections were males 16 (6.4%) and 8 (3.2%) were females. The reason for the predominance of male patients colonized with VRE infections could be due to the fact that, especially in rural settings, it is generally the males who seek medical attention as compared to females who tend to ignore their sufferings.

The rate of colonization with VRE was 12.5% (3/24) after 48 h which increased to 87.5% (21/24) after 5 days. Thus, our study reveals that the rate of colonization with VRE increased proportionately with increase in length of stay in the hospital, which is probably due to longer exposure to the hospital mileu which harbor these organisms.

Eighteen out of the 24 VRE colonized patients were found to receive various antibiotics, which had significant effect ($P = 0.001$) on VRE carriage. In another study, significant relation between previous administration of antibiotics and VRE carriage was reported ($P = 0.02$).[15]

Majority of the VRE positive isolates from clinical samples were from the age group 51–60 years, 9 (25.0%), which is expected as most VRE infections are found in immunosuppressed and debilitated patients. Some authors reported 12.7% VRE positive cases from the middle age group 46–60 years and 5.2% cases in the age group 61–75 years.[16] In yet another study, the minimum age of the patients with highly resistant *Enterococci* was 18 years and the maximum age was 71 years.[15] No significant relation between age and VRE colonization was noted by the authors. These differences may be due to different sample size.

Majority of the clinical isolates (both VRE and VSE) were recovered from urine 47.2% (118/250), followed by wound infection 31.6% (79/250), blood 13.6% (34/250), and catheter samples 7.6% (19/250), which is consistent with reports that *Enterococci* have become the leading cause of UTI, surgical wound infection, bacteramia, and catheter induced infection.[12] The bladder, prostate, and kidney are commonly infected by *Enterococci,* especially in patients with structural abnormalities of the urinary tract, indwelling catheters, or following instrumentation.

Our results were concordant with many Indian studies showing a gradual increase in resistance to penicillins and ampicillin over the years. For clinical VSE, maximum resistance was seen with penicillin 84.1% (180/214), followed by ampicillin 70.0% (150/214), and piperacillin 67.8% (145/214). For clinical VRE, maximum resistance was also seen with the same antibiotics viz.: Penicillin 100% (36/36), ampicillin 91.6% (33/36), and piperacillin 75.0% (27/36). Least resistance was seen with linezolid for both VSE and VRE, being 0%. Other authors have reported that clinical isolates of *Enterococci* showed maximum resistance to gentamicin (58.0%), followed by tetracycline (47.1%), and ampicillin (43.0%), with 0% resistance to vancomycin and linezolid.[17] In another study from Bangalore, maximum resistance was seen with erythromycin and least with teicoplanin and linezolid.[18]

CONCLUSION

This study reveals the emergence of vancomycin resistant *E. faecalis* isolates carrying *van A* gene and vancomycin resistant *E. gallinarum* with *van B* gene from this geographic region. This finding is alarming since it suggests the possibility of transfer of these plasmid borne *van A* and *van B* genes to other Gram-positive bacteria as well as to other plasmid free *Enterococci* both in the GIT and in hospital environment. It is, therefore, imperative to maintain a strict vigil on the spread of these organisms in the hospital and also from the hospital to the community.

The use of molecular methods reduces the errors associated with phenotypic disc diffusion methods. Standardization of the PCR results with phenotypic tests will enable a laboratory to choose an array of tests that needs to be performed in a particular laboratory in a particular area based on the requirement and infrastructure of the laboratory concerned.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Pourakbari B, Aghdam KM, Mahmoudi S, Ashtiani HTM, Sabouni F, Movahedi Z, *et al.* High frequency of vancomycin resistant Enterococcus faecalis in an Iranian referral children medical hospital. Am J Clin Med 2012;7:201-4.
2. Centers for Disease Control and Prevention (CDC) NNIS System. National nosocomial infection surveillance (NNIS) system report. Am J Infect Control 2000;28:429-48.
3. Desai PJ, Pandit D, Mathur M, Gogate A. Prevalence, identification and distribution of various species of *Enterococci* isolated from clinical specimens with special reference to urinary tract infection in catheterized patients. Indian J Med Microbiol 2001;19:132-7.
4. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC, editors. Gram-positive cocci Part 2: Streptococci, *Enterococci* and the *Streptococcus* like bacteria. In: Colour Atlas and Text Book of Diagnostic Microbiology. 6th ed. Philadelphia: Lippincott; 2006. p. 725-33.
5. Cetinkaya Y, Falk P, Mayhall CG. Vancomycin-resistant *Enterococci*. Clin Microbiol Rev 2000;13:686-707.
6. Betty AF, Daniel LS, Weissfeld AS, editors. Overview of bacterial identifications methods and strategies. In: Bailey & Scott's Diagnostic Microbiology. 12th ed. Missouri: Mosby Elsevier; 2007. p. 216-41.
7. Sahm DF, Free L, Smith C, Eveland M, Mundy LM. Rapid characterization schemes for surveillance isolates of vancomycin-resistant *Enterococci*. J Clin Microbiol 1997;35:2026-30.
8. CLSI. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-third Informational Supplement, M100-S23. Vol. 33. Wayne, USA;, CLSI; 2008. p. 90-3.
9. Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA, editors. Special Phenotypic Methods for Detecting Antibacterial Resistance. In: Manual of Clinical Microbiology. 9th ed. Washington, DC: ASM Press; 2007. p. 1152-72.
10. Vankerckhoven V, Van Autgaerden T, Vael C, Lammens C, Chapelle S, Rossi R, *et al.* Development of a multiplex PCR for the detection of asa1, gelE, cylA, esp, and hyl genes in *Enterococci* and survey for virulence determinants among European hospital isolates of *Enterococcus faecium*. J Clin Microbiol 2004;42:4473-9.
11. Sharifi Y, Hasani A, Ghotaslou R, Naghili B, Aghazadeh M, Milani M, *et al.* Virulence and antimicrobial resistance in *Enterococci* isolated from urinary tract infections. Adv Pharm Bull 2013;3:197-201.
12. Adhikari L. High-level Aminoglycoside resistance and reduced susceptibility to vancomycin in nosocomial *Enterococci*. J Glob Infect Dis 2010;2:231-5.
13. Littvik AM, López TN, González SE, Fernández CM, Pavan JV. Colonization with vancomycin-resistant *Enterococci* (VRE) in intensive care unit patients in Cordoba City, Argentina. Rev Argent Microbiol 2006;38:28-30.
14. Toledano H, Schlesinger Y, Raveh D, Rudensky B, Attias D, Eidelman AI, *et al.* Prospective surveillance of vancomycin-resistant *Enterococci* in a neonatal intensive care unit. Eur J Clin Microbiol Infect Dis 2000;19:282-7.
15. Javadi A, Ataei B, Khorvash F, Toghyani S, Mobasherzadeh S, Soghrati M. Prevalence of vancomycin resistant *Enterococci* colonization in gastrointestinal tract of hospitalized patients. Iran J Clin Infect Dis 2008;3:137-41.
16. Modi GB, Soni ST, Patel KJ, Goswami HM, Vegad MM. Prevalence of vancomycin resistant *Enterococci* in tertiaty care hospital, western India. Int J Microbiol Res 2012;4:182-5.
17. Bose S, Ghosh KA, Barapatre R. Prevalence of drug resistance among *Enterococcus* species isolated from a tertiary care hospital. Int J Med Health Sci 2012;1:38-44.
18. Fernandes SC, Dhanashree B. Drug resistance and virulence determinants in clinical isolates of *Enterococcus* species. Indian J Med Res 2013;137:981-5.

Virulence Markers of Vancomycin Resistant Enterococci Isolated from Infected and Colonized Patients

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ABSTRACT

Background: The aim of study was to find out the potential pathogenic role of virulence factors elaborated by strains of vancomycin resistant enterococci (VRE) isolated from clinical samples and VRE colonizing the gastrointestinal tract of hospitalized patients. **Materials and Methods:** Enterococci were isolated from various clinical samples and also from fecal specimens of colonized patients at the time of admission, after 48 h and after 5 days of admission. Various virulence determinants were detected by phenotypic tests. Vancomycin susceptibility in enterococci was detected by disc diffusion and agar screen method. Minimum inhibitory concentration was determined by agar dilution method. **Results:** Out of all the clinical and fecal samples processed, 12.0% isolates were either vancomycin resistant or vancomycin intermediate. Hemagglutinating activity against rabbit red blood cells was seen with 27.8% and 25.0% of clinical and fecal strains, respectively. Slime layer formation was seen with fecal VRE strains (37.5%) when compared to clinical VRE (27.8%). Among the clinical VRE strains the most prolific biofilm producers were *Enterococcus. fecalis* (92.9%) when compared to *Enterococcus. faecium* (52.9%). Biofilm formation/(presence of adhesions) was also seen in (29.2%) of the fecal VREs. In wound infection production of gelatinase, deoxyribonuclease (DNase), and caseinase (70.0% each) were the major virulence factors. The predominant virulence factors seen in the blood stream infection were adhesin, and hemolysin (44.4% each) and in catheter induced infection were DNase and adhesins (75.0% each). Adhesin (29.2%), slime layer (37.6%), DNAse (33.3%), gelatinase (25.0%), lipase (20.8%) and caseinase (16.6%) and hemolysin (8.3%) were produced the fecal isolates. **Conclusion:** An association between adhesin (as detected by biofilm formation) and urinary tract infection, adhesion and hemolysin with BSI, as also between DNase gelatinase & caseinase with wound infection was noted.

Key words: Blood stream infection, Catheter induced infection, Urinary tract infection, Vancomycin intermediate enterococci, Vancomycin resistant enterococci

INTRODUCTION

Enterococci are implicated in blood stream infections (BSI), endocarditis, urinary tract infections (UTI), pyogenic infections, intra-abdominal and pelvic infections.[1] Enterococci can infect humans because of its many virulence factors associated with biofilm formation including gelatinase, aggregation substance, capsule formation and enterococcal surface protein. Biofilms on medical devices favors disease sustenance because of restricted penetration of antimicrobials.[2-4]

Invasion is usually facilitated by damage to the host tissues and presence of bacterial virulence factors, which along with antibiotic resistance assist in advancement and further survival in newly infected places. Studies on adhesive properties of hemagglutinins produced by enterococci may contribute toward understanding the interaction of these organisms and the host cell surface and the mechanism of attachment.[5]

In recent years, an increase in the prevalence of *Enterococcus. faecium* has been seen which can be explained in part by the emergence of vancomycin resistant enterococci (VRE) and *Enterococcus. faecium* being the dominant detectable species among them.[6]

There is a paucity of information on the virulence factors distributed amongst enterococcal species.[7] The putative virulence markers in enterococcal strains isolated from various clinical sources and colonized patients and also the possible link between the presence of virulence factors and human infections was therefore investigated.

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room ohm. The colonies appeared mucoidal, runny or slimy due to production of polysaccharide.[12] — [Figure 2]

Deoxyribonuclease test

Test strains were inoculated on deoxyribonuclease (DNAse) agar (HiMedia, Mumbai, India). Clearing of the medium around the colonies indicated a positive test.[13]

Phosphatase test

Test strains were inoculated on phenolphlathelein phosphatase agar (HiMedia, Mumbai, India). The colonies turning pink on addition of ammonia solution were considered positive.[13]

Biofilm detection assa

The test strains were grown overnight at 37°C in Brain Heart Infusion broth (HiMedia, Mumbai,India) plus 0.25% glucose. Culture was diluted 1:20 in the same media. 200 µL of this suspension was used to inoculate sterile 96 well polystyrene microtitre plates. After 24 h at 37°C of static incubation, wells were washed with PBS, dried in inverted position and stained with 1% crystal violet for 15 min. The cells were rinsed once more and solubilized in 200 µl ethanol/acetone (80:20 v/v). The A_{630} was determined using microtitre plate reader. Biofilm formation was scored as non biofilm forming (−), weak- (+), moderate- (++), strong- (+++) corresponding to the A_{630} values ≤1, 1-≤2, 2-≤ 3 and > 3 respectively.[14]

Antimicrobial susceptibility & minimum inhibitory concentration tests

Antibiotic susceptibility test was done by Kirby-Bauer disc diffusion method on Muller-Hinton agar. Minimum inhibitoru concentration of VRE was determined by agar dilution method using the following concentration of vancomycin 0.5 µg/mL to 64 µg/mL. The test was quality controlled using *Enterococcus. fecalis* ATCC 51299 and *Enterococcus. fecalis* ATCC 29212.[15,16]

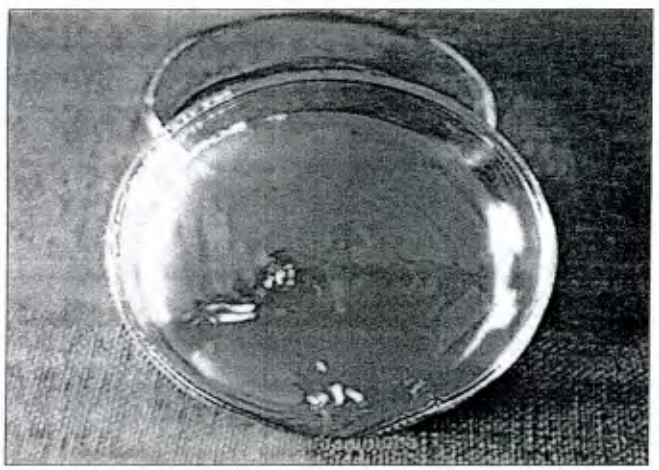

Figure 2: Slime layer formation

Statistical analysis

Statistical analysis was performed using chi-square test. $P < 0.005$ was considered significant in the results.

RESULTS

A total of 500 samples (250 each clinical and fecal) were processed out of which 60 (36 from clinical and 24 from fecal samples) were either vancomycin resistant or vancomycin intermediate enterococci (VIE). 25 (69.4%) VRE and 11 (30.5%) VIE were isolated from clinical samples and 12 (50.0%) VRE and VIE each were isolated from fecal samples.

Amongst the 36 clinical VRE/ VIE, 13 (36.1%) strains were isolated from cases of UTI. 10 (27.7%) strains were isolated from wound infection, 9 (25.0%) strains were isolated from BSI and 4 (11.1%) cases from catheter induced infection (CII). Most of these clinical VRE were found to be multidrug resistant.

A total of 9 (25.0%) clinical VRE/VIE were hemolytic to sheep red blood cells (RBC) as compared to 2 (8.3%) fecal VRE/VIE. This finding was found to be statistically insignificant (P = 0.102)-[Table 1]. The

Table 1: Comparative evaluation of the virulence factors in clinical and faecal VRE/VIE

Phenotypic virulence markers	Clinical VRE/VIE *n* =36 (%)	Faecal VRE/VIE *n* = 24 (%)	*P* value
Hemolysis of sheep RBC	9 (25.0)	2 (8.3)	0.102
Hemolysis of human RBC	9 (25.0)	2 (8.3)	0.102
Hemagglutination of rabbit RBC	10 (27.8)	6 (25.0)	0.812
Hemagglutination of human "O" RBC	9 (25.0)	5 (20.8)	0.709
Hemagglutination of human "B' RBC	9 (25.0)	5 (20.8)	0.709
Production of deoxyribonuclease	14 (38.9)	8 (33.3)	0.662
Slime layer	10 (27.8)	9 (37.6)	0.428
Lipase	4 (11.1)	9 (37.6)	0.015
Gelatinase	14 (38.9)	6 (25.0)	0.264
Caseinase	11 (30.6)	4 (16.6)	0.224
Adhesins (responsible for biofilm formation)	22 (61.1)	7 (29.2)	0.015

Statistically significant (*P* value < 0.005); VRE: Vancomycin resistant enterococci; VIE: Vancomycin intermediate enterococci; RBC: Red blood cells

Adhesin molecules (84.6%) were the predominant virulence marker among UTI isolates. None of the UTI isolates were found to produce lipase or caesinase. Gelatinase, DNase and caesinase 70.0% each were produced by the isolates from wound infection. DNase and adhesions (75.0% each) were elaborated by VRE causing CII. In BSI adhesions and hemolysins were the predominant virulence factors elaborated (44.4%)-[Table 5].

DISCUSSION

Correlation between the presence of virulence markers in the clinical and fecal VRE and VIE were evaluated. The presence of these virulence determinants in *Enterococcus. fecalis, Enterococcus. faecium* followed by *Enterococcus. gallinarum* is necessary for adhesion, tissue invasion and causing disease.

In this study, only 25.0% of clinical VRE and VIE isolates and 8.3% of fecal VRE and VIE isolates produced hemolysis around colonies on BHI sheep and human blood agar plates. Some authors have reported that 75.0% of clinical *E. faecalis* were hemolytic to sheep RBCs.[9] Another study found higher number of hemolysin producers in clinical isolates (60.0%) when compared to fecal isolates (17.0%).[17] These findings suggest a role of hemolysin in the causation of human disease.

Among the clinical VRE/VIE strains production of hemolysin was higher in *Enterococcus. fecalis* (28.5%) than in *Enterococcus. faecium* (23.5%). In another study only 16% *Enterococcus. fecalis* isolates were hemolysin producers and none of the *Enterococcus. faecium* strains were found to produce hemolysin. Hemolysin producing strains of *Enterococcus. fecalis* are virulent in animal models and human infections with these strains are associated with increased severity of illness.

Elaboration of virulence markers like hemolysin was most common in the isolates from BSI (44.4%) followed by CII (25.0%), wound infection (20.0%), UTI (15.4%) and colonized patients (8.3%). In contrast, in yet another study of *Enterococcus. fecalis,* only 16% of endocarditis isolates, 32% of blood culture isolates and 20% of community acquired fecal isolates were found to be hemolytic leading to the conclusion that hemolysin is not an essential factor in the pathogenicity of enterococci causing these infections.[18] However, findings of this study shows that a much higher number of VRE/VIE strains causing BSI were hemolysin producers when compared to VRE strains causing other infections. Hemolysin production has been associated with the better ability of enterococci to reach bloodstream to induce septicemia and with fivefold increased risk of adverse terminal outcome in patients with enterococcal bacteremia.[19]

HA activity against rabbit RBC was more (26.7%) than against human RBC (23.3%) in both clinical and fecal strains. Contrasting results were shown in another study in which 12.7% of enterococci isolated from humans agglutinated human RBC as compared to 4.8% of rabbit RBCs.[10] Another study reported that 97.0% of *Enterococcus. fecalis* strains isolated from human blood were hemagglutination-positive, while all of 24 *Enterococcus.*

Table 4: Biofilm formation in the VRE/VIE isolated from infected patients

	Biofilm formation in VRE/VIE isolated from clinical samples													
	E. faecalis, n = 14					*E. faecium, n* = 17					*E. gallinarum, n* = 5			
Biofilm forming capacity	UTI	Wound infection	Catheter induced infection	Blood infection	Total	UTI	Wound infection	Catheter induced infection	Blood infection	Total	UTI	Wound infection	Catheter induced infection	Blood infection
Weak	4	0	0	1	5	3	0	1	0	4	0	0	0	0
Moderate	2	2	2	1	7	2	0	0	2	4	0	0	0	0
Strong	0	0	1	0	1	0	0	1	0	1	0	0	0	0
Total	6	2	3	2	13	5	0	2	2	9	0	0	0	0

VRE: Vancomycin resistant enterococci; VIE: Vancomycin intermediate enterococci; UTI: Urinary tract infection; *E. faecalis: Enterococcus faecalis*; *E. faecium: Enterococcus faecium*; *E. gallinarum: Enterococcus gallinarum*

Table 5: Distribution of virulence markers in the VRE/VIE isolated from different types of infection

Type of infection/colonization	Adhesins (%)	Slime layer (%)	Hemolysin (%)	Gelatinase (%)	DNAse (%)	Lipase (%)	Caesinase (%)
UTI *n*=13	11 (84.6)	5 (38.5)	2 (15.4)	2 (15.4)	2 (15.4)	0	0
Wound infection *n*=10	4 (40.0)	3 (30.0)	2 (20.0)	7 (70.0)	7 (70.0)	3 (30.0)	7 (70.0)
BSI *n*=9	4 (44.4)	1 (11.1)	4 (44.4)	3 (33.3)	2 (22.2)	1 (11.1)	2 (22.2)
CII *n*=4	3 (75.0)	1 (25.0)	1 (25.0)	2 (50)	3 (75.0)	0	2 (50.0)
Total no of infections *n*=36	22 (61.1)*	10 (27.8)	9 (25.0)	14 (38.9)	14 (38.9)	4 (11.1)	11 (30.6)

Percentages calculated horizontally; *P = 0.015; VRE: Vancomycin resistant enterococci; VIE: Vancomycin intermediate enterococci; DNase: Deoxyribonuclease

adhesion. Enzyme treatment of bacterial suspensions, which led to partial loss of HA activity, suggests that the hemagglutinins are protein in nature. On the other hand heating method did not have any effect on bacteria-erythrocyte interactions. These observations lead us to conclude that the hemagglutinins are thermostable. The abundance of slime layer among the fecal isolates is particularly important in the acquisition of resistance by promoting cell- cell contact and the conjugal transfer of plasmids harbouring resistance and virulence genes. Such strains appear to represent the entry gateway to new resistance genes into enterococcal species in the gastrointestinal tract and may contribute to spreading of such bacteria in the hospital settings. Adhesin molecules (responsible for biofilm formation) were the main virulence factor of the UTI causing isolates. This strongly reflects the affinity of enterococcal isolates towards the urinary tract epithelial cells, and explains the prevalence of enterococci as a causative agent of nosocomially acquired UTIs. Gelatinase, DNAse and caseinase were the main virulence factors isolated from wound infection. Thus our findings suggest that these virulence factors collectively, may promote the spread of VRE in wound infections.

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REFERENCES

1. Murray BE. The life and times of the *Enterococcus*. Clin Microbiol Rev 1990;3:46-65.
2. Huycke MM, Sahm DF, Gilmore MS. Multiple Drug resistant Enterococci: The Nature of the Problem and Agenda for the future. Emerging infectious Diseases 1998;4:239-49.
3. Oli AK, Raju S, Rajeshwari, Nagaveni S, Kelmani CR. Biofilm formation by Multidrug resistant Enterococcus fecalis (MDEF) originated from clinical samples. J Microbiol Biotechnol Res 2012;2:284-8.
4. Toledo Arana A, Valle J, Solano C, Arrizubieta MJ, Cucarella C, Lamata M *et al*. The enterococcal surface protein, Esp is involved in *Enterococcus fecalis* biofilm formation. Appl Enviorn Microbiol 2001;67:4538-45.
5. Johnson AP. The pathogenecity of enterococci. J of Antimicrob Chemother 1994;33:1083-9.
6. Jahangiri S, Talebi M, Eslami G, Pourshafie MR. Prevalence of virulence factors and antibiotic resistance in vancomycin- resistant *Enterococcus faecium* isolated from sewage and clinical samples in Iran. Ind J of Med Microbiol 2010;28:337-41.
7. Laukkanen H, Kinnunen K, Mertaniemi V, Wright A, Oscorman virulence factors among human intestinal enterococcal isolates. Int J Appl Microbiol 2005;41:341-44.
8. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. Gram positive cocci Part 2: Streptococci, Enterococci and the Streptococcus like bacteria. In: Colour Atlas and Text book of Diagnostic Microbiology 6th ed. Philadelphia:Lippincott; 2006. p. 625-33.
9. Furumura MT, Figueiredo PM, Carbonell GV, Darini AL, Yano T. Virulence associated characteristics of *Enterococcus fecalis* strains isolated from clinical sources. Braz J Microbiol 2006;37:230-6.
10. Gulhan T, Aksakal A, Ekin HI, Savasan S, Boynukara B. Virulence factors of *Enterococcus fecium* and *Enterococcus fecalis* strains Isolated from Humans and Pets. Turk J Vet Anim Sci 2006;30:477-82.
11. Carvalho Mda G, Teixeira LM. Hemagglutination properties of *Enterococcus*. Current Microbiol 1995;30:265-68.
12. Betty AF, Daniel LS, Weissfeld AS, editors. Overview of Bacterial Identifications Methods and Strategies. In: Bailey & Scott's Diagnostic Microbiology. 12th ed. Mosby Elsevier, Missouri; 2007. p. 216-41.
13. Collee JG, Duguid JP, Fraser AG, Marmion BS Simmons A editors. Laboratory strategy in the diagnosis of infective syndromes. In: Mackie Mc Cartney Practical Medical Microbiology. 14th ed. Churchill livingstone; 2006. p. 113-29.
14. Prabakaran K, Aberna AR. Evaluation for the association of virulence determinants among *E. fecalis* with its clinical outcome. Int J of Biol Med Res 2011;2:523-27.
15. CLSI. Performance standards for antimicrobial susceptibility testing: Twenty-third informational supplement, M100-S23, January. Vol. 33 No. 1. CLSI; 2008. p. 90-93.
16. Murray PR, Baron EJ, Jorgensen JH, Landry ML and Pfaller MA, editors. Special Phenotypic Methods for Detecting Antibacterial Resistance. In : Manuel of Clinical Microbiology. 9th ed. Washington DC: ASM Press; 2007. p. 1152-72.
17. Ike Y, Hasimoto H & Clewell DB. High incidence of hemolysin production by *Enterococcus(Streptococcus) faecalis* strains associated with human parenteral infections. J Clin Microbiol 1987;25:1524-8.
18. Coque TM, Patterson JE, Steckelberg JM, Murray BE. Incidence of hemolysin, gelatinase and aggregation substance among enterococci isolated from patients with endocarditis and other infections and from feces of hospitalized and community-based persons. J Infect Dis 1995;171:1223-9.
19. Semedo T, Almeida Santos M, Martins P, Silva Lopes MF, Figueiredo Marques JJ, Tenreiro R, *et al*. Comparitive study using type strains and clinical and food isolates to examine hemolytic activity and occurance of the cyl operon in enterococci. J Clin Microbiol 2003;41:2569-76.
20. Elsner HA, Sobottka I, Mack D, Claussen M, Laufs R, Wirth R. Virulence factors of *Enterococcus faecalis* and *Enterococcus faecium* blood culture isolates . Eur J Clin Microbiol Infect Dis 2000;19:39-42.
21. Oli AK, Raju S, Rajeshwari D, Nagaveni. S, Chandrakanth .R. Biofilm formation by multidrug resistant *Enterococcus fecalis* originated from clinical samples. J Microbiol Biotech Res 2012;2:284-8.

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ORIGINAL ARTICLE

Molecular Characterization of Virulence Genes in Vancomycin-Resistant and Vancomycin-Sensitive Enterococci

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ABSTRACT

Background: The aim of this study was to find out the correlation between presence of virulence (gelatinase [*gel E*], enterococcal surface protein [*esp*], cytolysin A [*cyl A*], hyaluronidase [*hyl*], and aggregation substance [*asa1*]) and vancomycin-resistant genes (*van A* and *van B*) in enterococci, with their phenotypic expression. **Materials and Methods:** A total of 500 isolates (250 each clinical and fecal) were processed. Enterococci were isolated from various clinical samples and from fecal specimens of colonized patients. Various virulence determinants namely *asa1*, *esp*, *hyl*, *gel E*, and *cyl* were detected by phenotypic methods. Minimum inhibitory concentration (MIC) of vancomycin was determined by agar dilution method. Multiplex polymerase chain reaction (PCR) was used to detect the presence of virulence and *van* genes. **Results:** Out of all the samples processed, 12.0% (60/500) isolates carried *van A* or *van B* genes as confirmed by MIC test and PCR methods. Genes responsible for virulence were detected by multiplex PCR and at least one of the five was detected in all the clinical vancomycin-resistant enterococci (VRE) and vancomycin-sensitive enterococci (VSE). *gel E*, *esp*, and *hyl* genes were found to be significantly higher in clinical VRE. Of the fecal isolates, presence of *gel E*, *esp*, and *asa1* was significantly higher in VRE as compared to VSE. The presence of *hyl* gene in the clinical VRE was found to be statistically significant ($P = 0.043$) as against the fecal VRE. Correlation between the presence of virulence genes and their expression as detected by phenotypic tests showed that while biofilm production was seen in 61.1% (22/36) of clinical VRE, the corresponding genes, i.e., *asa1* and *esp* were detected in 30.5% (11/36) and 27.8% (10/36) of strains only. **Conclusion:** *Enterococcus faecium* isolates were found to carry *esp* gene, a phenomenon that has been described previously only for *Enterococcus faecalis*, but we were unable to correlate the presence of *esp* with their capacity to form biofilms.

Key words: Aggregation substance, cytolysin A, enterococcal surface protein, gelatinase, hyaluronidase

INTRODUCTION

Enterococcus species are now recognized as important causes of urinary tract infections, postsurgical wound infections, bacteremia, endocarditis, meningitis, neonatal sepsis, and infections in transplant patients with *Enterococcus faecalis* and *Enterococcus faecium* responsible for the majority of these infections.[1] Nevertheless, the incidence of other species of enterococci from clinical sources shows an alarming increase. This is attributable to their acquisition of various putative virulence determinants and multidrug resistance.[2]

A number of genes encoding for virulence factors including aggregation substance (*asa1*), enterococcal surface protein (*esp*), hyaluronidase (*hyl*), gelatinase (*gel E*), and cytolysin (*cyl*) in *E. faecalis* and *E. faecium* have been described and their effects have been shown in human and animal studies.[3] *asa1*, a surface protein adhesin encoded by the gene *asa1* has a contribution to virulence together with cyl. It facilitates the aggregation of the donor and recipient bacteria for efficient transfer of transmissible

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medium and incubated at 37°C for 24-48 h. Positive test result was read as formation of thin iridescent pearly layer overlying the colonies and a confined opalescence in the medium, which was seen when the colonies were scraped off.[12]

Slime layer formation

Brain heart infusion agar (Hi-Media, Mumbai, India) supplemented with 5% sucrose was used to determine the ability of *Enterococcus* species to produce extracellular polysaccharide on the agar. Test strains grown in Todd-Hewitt broth (Hi-Media, Mumbai, India) were used as the inoculum. The colonies appeared mucoidal, runny, or slimy due to the production of polysaccharide.[13]

Deoxyribonuclease test

Test strains were inoculated on deoxyribonuclease agar (Hi-Media, Mumbai, India). Clearing of the medium around the colonies indicated a positive test.[17]

Biofilm detection assay

The test strains were grown overnight at 37°C in Brain Heart Infusion broth (Hi-Media, Mumbai, India) plus 0.25% glucose. Culture was diluted 1:20 in the same media. 200 µL of this suspension was used to inoculate sterile 96 well polystyrene microtiter plates. After 24 h at 37°C of static incubation, wells were washed with PBS, dried in inverted position, and stained with 1% crystal violet for 15 min. The cells were rinsed once more and solubilized in 200 µl ethanol/acetone (80:20 v/v). The A_{630} was determined using microtiter plate reader. Biofilm formation was scored as nonbiofilm forming (−), weak - (+), moderate - (++), and strong - (+++) corresponding to the A_{630} values ≤1, 1-≤2, 2-≤3, and >3, respectively.[18]

Antimicrobial susceptibility and minimum inhibitory concentration tests

Antibiotic susceptibility test was done by Kirby-Bauer disc diffusion method on Mueller-Hinton agar. Minimum inhibitory concentration (MIC) of VRE was determined by agar dilution method using the following concentration of vancomycin 0.5-64 µg/mL. The test was quality controlled using *E. faecalis* ATCC 51299 and *E. faecalis* ATCC 29212.[19,20]

Protocol for multiplex polymerase chain reaction

DNA extraction method

Genomic DNA used as template for polymerase chain reaction (PCR) amplification was prepared using conventional phenol-chloroform DNA extraction method. PCR was performed in PCR system, model number T1 Thermoblock. The oligonucleotide primer pairs used to amplify the virulence genes *asa1*, *cyl A*, *gel E*, *esp*, and *hyl* as well as the vancomycin-resistant genes *van A*, *van B*, and the expected amplicon sizes are as follows [Table 1]:

The amplification of virulence genes was carried out as follows: Initial denaturation at 95°C for 15 min followed by denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min. The PCR amplification of the *van* genes were carried out as follows: Predenaturation at 95°C for 4 min followed by denaturation at 95°C for 30 cycles of 30 s each; 1 min for annealing at 52°C and elongation at 72°C for 1 min. Both positive control and negative control, consisting solely of the PCR reaction mixture without DNA template were included to check the validity of the technique utilized in the study.[21,22]

Analysis of DNA by agarose gel electrophoresis

Twenty-five microliters of respective amplified products were loaded into the wells and electrophoresed at a constant current of 50V for about 45 min using 1.5% agarose gel. A 100 bp DNA ladder marker was included as the standard molecular weight marker. The electrophoresed gel was later subjected to ethidium bromide staining and photographed under UV transillumination [Figures 1-3].

Statistical analysis

Statistical analysis was performed by Chi-square test. $P \leq 0.05$ was considered to be significant and $P \leq 0.001$ was considered to be highly significant. All statistical analyses

Table 1: Polymerase chain reaction primers and products for the detection of virulent genes and vancomycin-resistant genes

asa1	ASA11-GCACGCTATTACGAACTATGA ASA12-TAAGAAAGAACATCACCACGA	375
		600
		100
	GCACCCGAAATAATATA	
esp		570
hyl		200
	F14-CATGAATAGAATAAAAGTTGCAATA R14-CCCCTTTAACGCTAATACGATCAAA	1030
	F15-GTGACAAACCGGAGGCGAGGA R15-CCGCCATCCTCCTGCAAAAAA	433

***asa1*: Aggregation substance; *cyl A*: Cytolysin A; *gel E*: Gelatinase; *esp*: Enterococcal surface protein; *hyl*: Hyaluronidase**

Table 2: Comparative evaluation of the virulence factors in clinical and fecal isolates of vancomycin-sensitive enterococci and vancomycin-resistant enterococci

	P	*P*
isolates		
		0.096
fi		

P ≤ 0.05 or ≤0.001 was considered statistically significant; VSE: Vancomycin-sensitive enterococci; VRE: Vancomycin-resistant enterococci; RBC: Red blood cell

Table 3: Presence of virulence genes in vancomycin-sensitive enterococci and vancomycin-resistant enterococci (clinical and fecal)

	P	*P*	*P*
markers			
esp			
hyl			
asa1			

P ≤ 0.05 or ≤0.001 was considered statistically significant; VSE: Vancomycin-sensitive enterococci; VRE: Vancomycin-resistant enterococci; *gel E*: Gelatinase; *asa1*: Aggregation substance; *esp*: Enterococcal surface protein; *cyl A*: Cytolysin A

Table 4: Distribution of virulence genes in clinical vancomycin-resistant enterococci and vancomycin-sensitive enterococci

ffi
n=20

gel E: Gelatinase; *asa1*: Aggregation substance; *esp*: Enterococcal surface protein; *cyl A*: Cytolysin A; VSE: Vancomycin-sensitive enterococci; VRE: Vancomycin-resistant enterococci

only the presence of *hyl* gene was found to be significantly higher in clinical VRE than fecal VRE ($P = 0.043$) [Table 3].

Table 4 shows the presence of various virulence genes in VRE species. The predominant genes in *E. faecalis* were *gel E* 57.1%; followed by *asa1* 50.0%, *cyl A* and *esp* being 28.6% each, and *hyl* 21.4%. The predominant gene in *E. faecium* was *hyl* gene (53.0%) followed by *gel E*, *esp* 35.3% each, *cyl A*, and *asa1* 23.5% each. *Enterococcus gallinarum* showed the presence of *gel E* 40.0% followed by *cyl A* and *hyl* 20.0% each.

Out of the 14 VR *E. faecalis*, all the strains produced one or two virulence genes. Twelve (85.7%) strains of *E. faecalis* produced two virulence genes: 35.7% (5/14) strains showed the concomitant presence of *gel E* and *asa*, another 21.4% (3/14) strains expressed *gel A* and *hyl* genes; and 28.6% (4/14) strains presented *cyl A* and *esp* genes. Of the 17 VR *E. faecium*, 12 (70.6%) strains were found to produce two genes. The various combination of two virulence genes seen in *E. faecium* were 35.3% (6/17) expressed *gel A* with *hyl*, 17.6% (3/17), each, expressed *cyl A* with *esp* and *asa1* with *esp*.

Of the clinical VSE, the predominant genes in *E. faecalis* were *hyl* (28.0%); followed by *gel E* (26.0%), *esp* and *asa1* (22.0%) each, and *cyl A* (16.0%). *hyl* (46.5%) was the main gene in *E. faecium* followed by *asa1* (20.9%), *esp* (18.6%), *gel E* (16.3%), and *cyl A* (13.9%). Apart from the *esp* gene, the remaining four virulence genes were detected in all the nonfaecalis and nonfaecium strains. *Enterococcus durans* was the least virulent among VSEs as it showed the expression of only *cyl A* and *hyl* (5.9%) each [Table 4].

by phenotypic tests was more than the number of strains showing the presence of the corresponding genes by PCR namely *asa1* and *esp*.

DISCUSSION

Very few reports regarding the distribution of virulence genes in various species of enterococci from clinical samples are available.[23] This study was designed to identify various virulence genes in VRE and VSE as well as to evaluate the correlation between virulence markers and virulence genes both phenotypically and genotypically.

Only *gel E*, *esp*, and *hyl* genes were found to be significantly higher in clinical VRE than VSE. As for fecal isolates, presence of *gel E*, *esp*, and *asa1* was significantly higher in VRE than VSE. Other studies show that prevalence of *esp* ($P = 0.001$) and *hyl* ($P = 0.04$) genes were significantly higher among VRE isolates (44.4% and 27.7%) than among VSE isolates (16.4% and 8.8%).[24]

Authors reported *esp* of 80.0% (32/40) to be the predominant virulence factor followed by *gel E* of 50.0% (20/40) among VRE. Considering vancomycin resistance as variable, the authors did not find any significant difference in the presence of activity of virulence factors between resistant and susceptible enterococci.[25]

It has been reported that the *esp* gene has been restricted to vancomycin-resistant strains.[26] In contrast, our result showed the presence of *esp* gene in both VSE and VRE strains. The presence of *esp* in isolates susceptible and resistant to different antibiotics indicate that this trait probably emerged prior to the acquisition of resistance not only to vancomycin but also to other antibiotics used in hospital settings.

Of the clinical VRE, all the five virulence genes were detected in *E. faecalis* and *E. faecium*. However, virulence genes namely *asa1* and *esp* were absent in *E. gallinarum*. Until recently, a majority of the infection derived isolates were *E. faecalis* strains and was regarded as the most pathogenic species. It is known that *Enterococcus* possess highly efficient gene transfer mechanism. The virulent genes are associated with highly transmissible plasmids which might have led to the dissemination of virulent genes in less virulent *E. faecium* and *E. gallinarum*.

Of the VSE, all the five different virulence genes were detected in vancomycin-sensitive *E. faecalis* and *E. faecium*. The predominant genes in *E. mundtii*, *E. raffinosus*, *E. solitaries*, and *E. gallinarum* were *asa1* being 18.1%, 15.3%, 33.3%, and 28.6% (6/33, 4/28, 3/9, and 4/14), respectively. The higher prevalence of *hyl* gene in *E. faecalis* and *E. faecium* and *asa1* gene in nonfaecalis and nonfaecium strains from our setup depicts that this virulence marker may have permeated more deeply into the species by horizontal transfer and would have acquired it comparatively earlier, thereby enhancing the ability of the organism to cause disease beyond that intrinsic to the species. We observed a considerable number of *E. faecium* strains expressed *hyl* and *asa1* genes as compared to *esp*, *gel E*, and *cyl A* genes. At present, we cannot say with certainty whether, and to what extent, *E. faecium* actually makes *hyl*, and under what conditions this protein may be synthesized or exported. Northern hybridization experiments indicate that the *hyl* open reading frame is transcribed under nonselective growth conditions *in vitro*. Therefore, we have compelling reasons to believe that the protein is synthesized at least under some environmental conditions. *asa* too has an important effect on biofilm formation because this substance promotes the adherence of microorganisms to a surface.[27]

In another study, the distribution of virulence genes in various species was viz: *E. faecalis* 53.0% (35/66), *Enterococcus casseliflavus* 50.0% (2/4), *E. faecium* 40.6% (13/32), and *E. mundtii* 25.0% (1/4). *Enterococcus durans* showed the total absence of all virulene genes. *E. casseliflavus* and *E. mundtii* showed the presence of only two genes - *asa1* and *esp* 25.0% (1/4).[28] The permeation of these virulence genetic characteristics into different species differs according to the setup, patient demographics, and other extrinsic factors. Thus, the nonfaecalis and nonfaecium strains isolated from our study were more virulent as compared to the strains seen in the above study which may be due to the presence of *asa1* in nonfaecalis and nonfaecium strains that promote cell-to-cell contact and help in the transfer of other virulence genes through plasmids.

Of the clinical VRE, 85.7% (12/14) *E. faecalis* produced two virulence genes: 35.7% (5/14) strains showed the concomitant presence of *gel E* and *asa1*. Another 21.4% (3/14) strains expressed *gel E* and *hyl* genes and 28.6% (4/14) strains presented *cyl A* and *esp* genes. Of the 17 VR *E. faecium*, 12 (70.5%) strains were found to produce two genes. The various combination of two virulence genes seen in *E. faecium* were: 35.3% (6/17) *gel E* with *hyl*, 17.6% (3/17), each expressing *cyl A* with *esp*, and *asa1* with *esp*. Contrasting reports were seen in another study, where the most common combination of genes was between *asa1* and *gel E* from infections of hospitalized patients. This suggests that these traits entered the species earlier than did

5. Toledo-Arana A, Valle J, Solano C, Arrizubieta MJ, Cucarella C, Lamata M, *et al.* The enterococcal surface protein, Esp, is involved in *Enterococcus faecalis* biofilm formation. Appl Environ Microbiol 2001;67:4538-45.
6. Giridhara Upadhyaya PM, Ravikumar KL, Umapathy BL. Review of virulence factors of *Enterococcus*: An emerging nosocomial pathogen. Indian J Med Microbiol 2009;27:301-5.
7. Makinen PL, Clewell DB, An F, Makinen KK. Purification and substrate specificity of a strongly hydrophobic extracellular metalloendopeptidase (gelatinase) from *Streptococcus faecalis* (strain OG1-10). J Biol Chem 1989;264:3325-34.
8. Booth MC, Bogie CP, Sahl HG, Seizen RJ, Hatter KL, Gilmore MS. Structural analysis and proteolytic activation of *Enterococcus faecalis* cytolysin, a novel lantibiotic. J Mol Microbiol Biotechnol 1996;21:1175-84.
9. Fernandes SC, Dhanashree B. Drug resistance & virulence determinants in clinical isolates of *Enterococcus* species. Indian J Med Res 2013;137:981-5.
10. Ira P, Sujatha S, Chandra PS. Virulence factors in clinical and commensal isolates of *Enterococcus* species. Indian J Pathol Microbiol 2013;56:24-30.
11. Al Khafaji JK, Samaan SF, Al Saeed MS. Virulence factors of *Enterococcus faecalis*. Med J Babylon 2010;7:579-83.
12. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC, editors. Gram positive cocci. Part 2: Streptococci, enterococci and the streptococcus like bacteria. In: Colour Atlas and Text Book of Diagnostic Microbiology. 6th ed. Philadelphia: Lippincott; 2006. p. 725-33.
13. Betty AF, Daniel LS, Weissfeld AS, editors. Overview of bacterial identifications methods and strategies. In: Bailey & Scott's Diagnostic Microbiology. 12th ed. Missouri: Mosby Elsevier; 2007. p. 216-41.
14. Furumura MT, Figueiredo PM, Carbonell GV, Darini AL, Yano T. Virulence associated characteristics of *Enterococcus faecalis* strains isolated from clinical sources. Braz J Microbiol 2006;37:230-6.
15. Gulhan T, Aksakal A, Ekin IH, Savasan S, Boynukara B. Virulence factors of *Enterococcus fecium* and *Enterococcus feacalis* strains isolated from humans and pets. Turk J Vet Anim Sci 2006;30:477-82.
16. Carvalho Mda G, Teixeira LM. Hemagglutination properties of *Enterococcus*. Curr Microbiol 1995;30:265-8.
17. Collee JG, Duguid JP, Fraser AG, Marmion BS, Simmons A, editors. Laboratory strategy in the diagnosis of infective syndromes. In: Mackie and McCartney Practical Medical Microbiology. 14th ed. New York: Churchill Livingstone; 2006. p. 113-29.
18. Prabakaran K, Aberna AR. Evaluation for the association of virulence determinants among *E. fecalis* with its clinical outcome. Int J Biol Med Res 2011;2:523-7.
19. CLSI. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-third Informational Supplement. M100-S23. Vol. 33. Wayne, PA, USA: CLSI; 2013. p. 90-3.
20. Sapkota AR, Hulet RM, Zhang G, McDermott P, Kinney EL, Schwab KJ, *et al.* Lower prevalence of antibiotic-resistant enterococci on U.S. conventional poultry farms that transitioned to organic practices. Environ Health Perspect 2011;119:1622-8.
21. Vankerckhoven V, Van Autgaerden T, Vael C, Lammens C, Chapelle S, Rossi R, *et al.* Development of a multiplex PCR for the detection of asa1, gelE, cylA, esp, and hyl genes in enterococci and survey for virulence determinants among European hospital isolates of *Enterococcus faecium*. J Clin Microbiol 2004;42:4473-9.
22. Akhi TM, Farzaneh F, Oskouei M. Study of enterococcal susceptibility patterns isolated from clinical specimens in Tabriz, Iran. Pak J Med Sci 2009;25:1-7.
23. Anupurba S, Banerjee T. Drug resistance in clinical isolates of enterococci with special reference to vancomycin, from North India. J Pure Appl Microbiol 2012;6:807-12.
24. Duprè I, Zanetti S, Schito AM, Fadda G, Sechi LA. Incidence of virulence determinants in clinical *Enterococcus faecium* and *Enterococcus faecalis* isolates collected in Sardinia (Italy). J Med Microbiol 2003; 52(Pt 6):491-8.
25. Comerlato BC, Resende CC, Caierao J, Azevedo AP. Presence of virulence factors in Enterococcus faecalis and Enterococcus faecium susceptible and resistant to vancomycin. Int J Antimicrob Agents 2013;108:590-5.
26. Kafil SK, Ashgarzadi M. Vancomycin-resistant Enterococcus faecium and Enterococcus faecalis isolated from education hospital of Italy. J Med Microbiol 2013;9:323-7.
27. Trevedi K, Cupakova S, Karpiskova R. Virulence factors and antibiotic resistance in enterococci isolated from food-stuffs. J Vet Med 2012; 56:352-7.
28. Bittencourt de Marques E, Suzart S. Occurrence of virulence-associated genes in clinical *Enterococcus faecalis* strains isolated in Londrina, Brazil. J Med Microbiol 2004;53:1069-73.
29. Poeta P, Costa D, Rodrigues J, Torres C. Antimicrobial resistance and the mechanisms implicated in faecal enterococci from healthy humans, poultry and pets in Portugal. Int J Antimicrob Agents 2006;27:151-7.
30. Revathy S, Sridharan KS, Elumalai AS, Umasekar. Phenotypic detection of high-level aminoglycoside resistance (HLAR) in *Enterococcus* species in a tertiary care centre. J Biosci Biotechnol Res 2010;6:751-6.

5. Toledo-Arana A, Valle J, Solano C, Arrizubieta MJ, Cucarella C, Lamata M, *et al.* The enterococcal surface protein, Esp, is involved in *Enterococcus faecalis* biofilm formation. Appl Environ Microbiol 2001;67:4538-45.
6. Giridhara Upadhyaya PM, Ravikumar KL, Umapathy BL. Review of virulence factors of *Enterococcus*: An emerging nosocomial pathogen. Indian J Med Microbiol 2009;27:301-5.
7. Makinen PL, Clewell DB, An F, Makinen KK. Purification and substrate specificity of a strongly hydrophobic extracellular metalloendopeptidase (gelatinase) from *Streptococcus faecalis* (strain OG1-10). J Biol Chem 1989;264:3325-34.
8. Booth MC, Boogie CP, Sahl HG, Seizen RJ, Hatter KL, Gilmore MS. Structural analysis and proteolytic activation of *Enterococcus faecalis* cytolysin, a novel lantibiotic. J Mol Microbiol Biotechnol 1996;21:1175-84.
9. Fernandes SC, Dhanashree B. Drug resistance & virulence determinants in clinical isolates of *Enterococcus* species. Indian J Med Res 2013;137:981-5.
10. Ira P, Sujatha S, Chandra PS. Virulence factors in clinical and commensal isolates of *Enterococcus* species. Indian J Pathol Microbiol 2013;56:24-30.
11. Al Khafaji JK, Samaan SF, Al Saeed MS. Virulence factors of *Enterococcus faecalis*. Med J Babylon 2010;7:579-83.
12. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC, editors. Gram positive cocci. Part 2: Streptococci, enterococci and the streptococcus like bacteria. In: Colour Atlas and Text Book of Diagnostic Microbiology. 6th ed. Philadelphia: Lippincott; 2006. p. 725-33.
13. Betty AF, Daniel LS, Weissfeld AS, editors. Overview of bacterial identifications methods and strategies. In: Bailey & Scott's Diagnostic Microbiology. 12th ed. Missouri: Mosby Elsevier; 2007. p. 216-41.
14. Furumura MT, Figueiredo PM, Carbonell GV, Darini AL, Yano T. Virulence associated characteristics of *Enterococcus faecalis* strains isolated from clinical sources. Braz J Microbiol 2006;37:230-6.
15. Gulhan T, Aksakal A, Ekin IH, Savasan S, Boynukara B. Virulence factors of *Enterococcus feicium* and *Enterococcus faecalis* strains isolated from humans and pets. Turk J Vet Anim Sci 2006;30:477-82.
16. Carvalho Mda G, Teixeira LM. Hemagglutination properties of *Enterococcus*. Curr Microbiol 1995;30:265-8.
17. Collee JG, Duguid JP, Fraser AG, Marmion BS, Simmons A, editors. Laboratory strategy in the diagnosis of infective syndromes. In: Mackie and McCartney Practical Medical Microbiology. 14th ed. New York: Churchill Livingstone; 2006. p. 113-29.
18. Prabakaran K, Aberna AR. Evaluation for the association of virulence determinants among *E. fecalis* with its clinical outcome. Int J Biol Med Res 2011;2:523-7.
19. CLSI. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-third Informational Supplement, M100-S23. Vol. 33. Wayne, PA, USA: CLSI; 2013. p. 90-3.
20. Sapkota AR, Hulet RM, Zhang G, McDermott P, Kinney EL, Schwab KJ, *et al.* Lower prevalence of antibiotic-resistant enterococci on U.S. conventional poultry farms that transitioned to organic practices. Environ Health Perspect 2011;119:1622-8.
21. Vankerckhoven V, Van Autgaerden T, Vael C, Lammens C, Chapelle S, Rossi R, *et al.* Development of a multiplex PCR for the detection of asa1, gelE, cylA, esp, and hyl genes in enterococci and survey for virulence determinants among European hospital isolates of *Enterococcus faecium*. J Clin Microbiol 2004;42:4473-9.
22. Akhi TM, Farzaneh F, Oskouei M. Study of enterococcal susceptibility patterns isolated from clinical specimens in Tabriz, Iran. Pak J Med Sci 2009;25:1-7.
23. Anupurba S, Banerjee T. Drug resistance in clinical isolates of enterococci with special reference to vancomycin, from North India. J Pure Appl Microbiol 2012;6:807-12.
24. Duprè I, Zanetti S, Schito AM, Fadda G, Sechi LA. Incidence of virulence determinants in clinical *Enterococcus faecium* and *Enterococcus faecalis* isolates collected in Sardinia (Italy). J Med Microbiol 2003; 52(Pt 6):491-8.
25. Comerlato BC, Resende CC, Caierao J, Azevedo AP. Presence of virulence factors in Enterococcus faecalis and Enterococcus faecium susceptible and resistant to vancomycin. Int J Antimicrob Agents 2013;108:590-5.
26. Kafil SK, Ashgarzadi M. Vancomycin-resistant Enterococcus faecium and Enterococcus faecalis isolated from education hospital of Italy. J Med Microbiol 2013;9:323-7.
27. Trevedi K, Cupakova S, Karpiskova R. Virulence factors and antibiotic resistance in enterococci isolated from food-stuffs. J Vet Med 2012; 56:352-7.
28. Bittencourt de Marques E, Suzart S. Occurrence of virulence-associated genes in clinical *Enterococcus faecalis* strains isolated in Londrina, Brazil. J Med Microbiol 2004;53:1069-73.
29. Poeta P, Costa D, Rodrigues J, Torres C. Antimicrobial resistance and the mechanisms implicated in faecal enterococci from healthy humans, poultry and pets in Portugal. Int J Antimicrob Agents 2006;27:151-7.
30. Revathy S, Sridharan KS, Elumalai AS, Umasekar. Phenotypic detection of high-level aminoglycoside resistance (HLAR) in *Enterococcus* species in a tertiary care centre. J Biosci Biotechnol Res 2010;6:751-6.

REPRODUCTIVE TRACT INFECTION IN WOMEN ATTENDING OBSTETRICS AND GYNAECOLOGY DEPARTMENT OF A TERTIARY CARE HOSPITAL IN BIHAR

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ABSTRACT

BACKGROUND

Reproductive tract infection (RTI) is a common problem among women and represents a threat to their health. RTI including sexually transmitted disease (STD) and HIV/ AIDS are increasingly being recognised as a serious public health problem. The most common long-term sequelae are pelvic inflammatory disease (PID), cervical cancer, infertility, spontaneous abortion and ectopic pregnancy, which may lead to maternal death. The common infective agents are *Gardnerella vaginalis, Trichomonas vaginalis, Candida* species and *Neisseria gonorrhoeae*. Agents such as toxoplasma, rubella, CMV and HSV are important causes of infections during pregnancy. Most of the TORCH infections cause mild maternal morbidity, but have serious foetal consequences.

MATERIALS AND METHODS

Study Design**-** This prospective observational study was conducted in the Department of Microbiology, in a tertiary care medical college hospital in eastern Bihar. A total of 200 high vaginal swabs were collected in duplicate from an equal number of patients attending Obstetrics and Gynaecology Department with symptoms suggestive of RTI. In the laboratory one swab was used for culture and identification of the organism, the other was used for direct microscopic examination (Gram stain and wet mount) and other tests like amine test and measurement of pH.

RESULTS

Maximum patients were seen in the age group of 21 - 30 years, 43.5% (87/200). Least number of cases were seen in the age group of < 20 years 2.5% (5/200). Out of the 200 samples processed in the laboratory, 88 were found to show no growth. The most common isolate was *Candida* species, 25.5% (51/200). *Neisseria gonorrhoeae* 0.5% (1/200) was the least common organism isolated. Moreover, the overall rate of isolation of organisms was higher in patients with bad obstetric history.

CONCLUSION

Vulvovaginal candidiasis was the most common RTI followed by trichomoniasis and bacterial vaginosis. A single case of gonorrhoea was detected. Interestingly, the incidence of RTI was higher in patients with bad obstetric history.

KEYWORDS

Reproductive Tract Infection, Pelvic Inflammatory Disease, Bad Obstetric History.

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BACKGROUND

Reproductive tract infection (RTI) is a common problem among women and represents a threat to their health. Various socio-economic factors are responsible for the development of this disease entity, especially in a country like ours. Infection in the vulva, vagina or cervix represents lower reproductive tract infection and infection in uterus, fallopian tubes and ovaries are considered as upper reproductive tract infection.[1]

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RTI including sexually transmitted disease (STD) and

RTI including sexually transmitted disease (STD) and HIV/ AIDS are increasingly being recognised as a serious public health problem. The poor health of Indian women is a concern at both national and individual level. Women are not only more susceptible than men to these infections, but also are more prone to develop complications because infection in women is difficult to diagnose and therefore more likely to go untreated.[2]

RTIs often cause discomfort and lost economic productivity. The most common long-term sequelae are pelvic inflammatory disease (PID), cervical cancer, infertility, spontaneous abortion and ectopic pregnancy which may lead to maternal death. The presence of a sexually transmitted infection increases the risk of acquiring and transmitting HIV infection by three to five times and bacterial vaginosis may be a cofactor for HIV transmission, especially among younger women.[3]

The common infective agents are *Gardnerella vaginalis, Trichomonas vaginalis, Candida* species and *Neisseria*

gonorrhoeae. Agents such as Toxoplasma, rubella, CMV and HSV are important causes of infections during pregnancy.

Most of the TORCH infections cause mild maternal morbidity, but have serious foetal consequences.[4]

It is well realised that at least 12% - 15% of all recognised conceptions end in miscarriage and pre-clinical pregnancy loss rate is still higher (22% - 30%). At present, there is no implicating evidence that bacterial and fungal infections can cause recurrent abortions.[5] Studies have shown that these infections predispose the patients to acquisition of viral infections like HSV-2 and HIV, which in turn can lead to premature birth and spontaneous abortions.[6]

Reproductive tract infection is the commonest cause of morbidity in the reproductive age group, but the actual magnitude of the problem is grossly underestimated due to serious problems like social stigma. It is therefore imperative that proper initiation must be taken by health care providers to facilitate the diagnosis and treatment of such cases.

The present study was taken up with a view to determining the spectrum of microorganisms responsible for causing reproductive tract infection in our hospital setting and to find a possible correlation between their isolation pattern and obstetric history of the patient.

MATERIALS AND METHODS

This prospective observational study was carried out in a tertiary care medical college hospital in eastern Bihar. Institutional Ethical Committee clearance was taken before conducting the study. A total of 200 high vaginal swabs were collected in duplicate from an equal number of patients attending Obstetrics and Gynaecology Department with symptoms suggestive of RTI. A brief clinical history regarding occupation, personal hygiene etc. was noted. Obstetric history and history of any antibiotic uptake either topically or systemically was also noted down. Swabs collected were immediately sent to the laboratory for further processing.

In the laboratory one swab was used for culture and identification of the organism, the other was used for direct microscopic examination (Gram stain and wet mount) and other tests like Whiff test (amine test) and measurement of pH.[7]

Gram stained smears were further scored as per scoring system of Nugent et al. A score of ≥ 7 was indicative of bacterial vaginosis, score of 4 - 6 was taken as intermediate and score of 0 - 3 was considered as normal.[8]

Identification of the organisms isolated was done by study of colony morphology, Gram's staining and motility followed by a battery of biochemical tests.[7,9]

Neisseria gonorrhoeae was identified by colony morphology on chocolate agar, Gram's stain morphology, catalase, oxidase and rapid carbohydrate utilisation tests.[7]

Gardnerella vaginalis was identified on the basis of presence of β-haemolytic colonies on human blood Tween 80 bi-layer medium, presence of Gram variable bacilli on Gram staining, starch and raffinose fermentation and hippurate hydrolysis tests.[8]

Candida species was identified by germ tube test, chlamydospore formation on cornmeal agar, sugar fermentation and sugar assimilation test.[7]

Statistical analysis of results was done using the chi-square test. P-value ≤ 0.05 was considered to be significant and p-value ≤ 0.001 was considered to be highly significant. All statistical analysis was carried out using online statistical software at-

http://www.physics.csbju.edu/stats/contingency_NROW_NCOLUMN_form.html; accessed on 14.02.2018. All media and reagents were procured from HiMedia Laboratories, Mumbai.

RESULTS

A total of 200 females were inducted into the study, out of which maximum number of patients were seen in the age group of 21 - 30 years, 43.5% (87/200) followed by age group 31 - 40 years, 39.0% (78/200). Least number of cases were seen in the age group of < 20 years 2.5% (5/200) [Table 1].

Out of 200 women, 84.5% (169/200) were multiparous, only 15.5% (31/200) were nulliparous. Among the multiparous women 157 had a good obstetric history (GOH), while 12 had a bad obstetric history (BOH) [Table 2].

On microscopic examination of vaginal swabs 3% (6/200) patients showed presence of clue cells, 10% (20/200) showed presence of *Trichomonas sp.* and yeast-like cells were seen in 31% (62/200) of samples [Table 3].

Overall, RTI was seen in 48.5% (97/200) of patients in the study group, whereas 44.0% (88/200) showed no growth. The most common isolate was Candida species 25.5% (51/200) followed by non-albicans candida species 5.5% (11/200) and *Gardnerella vaginalis* 3% (6/200). *Neisseria gonorrhoeae* 0.5% (1/200) was the least common organism isolated. *Trichomonas vaginalis* could be detected by wet mount examination in 10% (20/200) of patients. The overall rate of isolation of organisms was higher in patients with BOH. The differences in rate of isolation of various organisms in patients with GOH, BOH and nullipara was found to be highly significant (p= 0.000) [Table 4].

As per Nugent criteria, 7% (14/200) of patients were found to have bacterial vaginosis. Out of these 14 patients, both Whiff test and vaginal pH ≥ 6 was found to be positive in 85.7% (12/14) and *Gardnerella vaginalis* could be grown in only 42.9% (6/14) of cases [Table 5].

Age	No. of Married Patients (%)	No. of Unmarried Patients (%)	Total
≤ 20	2 (1.0)	3 (100.0)	5 (2.5)
21-30	87 (44.2)	0	87 (43.5)
31-40	78 (39.6)	0	78 (39.0)
41-50	30 (15.2)	0	30 (15.0)
Total	**197**	**3**	**200**

Table 1. Age-Wise Distribution of Married and Unmarried Patients

Parity	No. of Patients	Percentage
Multiparous with good obstetric history	157	78.5
Multiparous with bad obstetric history	12	6.0
Nulliparous	31*	15.5
Total	**200**	**100.0**

Table 2. Obstetric History of Patients with RTI

*All married and unmarried women who never conceived were included in nullipara.

Diagnostic Procedure	Clue Cells (%)	Trophozoites of Trichomonas (%)	Yeast-Like Organisms (%)	No. Relevant Findings (%)
Wet-mount (n= 200)	6 (3.0)	20 (10.0)	62 (31.0)	112 (56.0)
Gram stain (n= 200)	10 (5.0)	NA	62 (31.0)	128 (64.0)

Table 3. Microscopic Findings in Patients with RTI

Organisms Isolated/Detected	Multiparous with Good Obstetric History (%)*	Multiparous with Bad Obstetric History (%)*	Nulliparous (%)*	Total No. (%)
Candida albicans	44 (28.0)	4 (33.3)	3 (9.7)	51 (25.5)
Non-albicans Candida species	8 (5.1)	2 (16.7)	1 (3.2)	11 (5.5)
Gardnerella vaginalis‖	5 (3.2)	1 (8.3)	0 (0.0)	6 (3.0)
*Trichomonas vaginalis***	17 (10.8)	3 (25.0)	0 (0.0)	20 (10.0)
Neisseria gonorrhoea	0 (0.0)	1 (8.3)	0 (0.0)	1 (8.3)
Growth of normal flora	17 (10.8)	1 (8.3)	5 (16.1)	23 (11.5)
Sterile	66 (42.0)	0 (0.0)	22 (71.0)	88 (44.0)
Total*	**157**	**12**	**31**	**200**

Table 4. Isolation Pattern of Different Organisms in Patients with RTI

- ‖isolated in only 6 of the 14 cases of bacterial vaginosis **detected by microscopy only.
- *p value= 0.000.

Patients with Bacterial Vaginosis (n= 14)	Whiff Test	Vaginal pH			Nugent Scoring			Growth of Gardnerella Vaginalis
		pH ≤ 4	pH 4-6	pH ≥ 6	0-3	4-6	≥ 7	
No. of Positive Cases (%)	12 (85.7)	00	02 (14.3)	12 (85.7)	00	00	14 (100.0)	06 (42.9)
No. of Negative Cases	02 (14.3)	14 (100.0)	12 (85.7)	02 (14.3)	14 (100.0)	14 (100.0)	00	08 (57.1)

Table 5. Positivity of Various Tests in Patients with Bacterial Vaginosis

DISCUSSION

Majority of the patients (43.5%) were in the age group of 21-30 years, followed by the age group of 31 - 40 years (39%). Least number of patients (2.5%) belonged to the age group of ≤ 20 years. This is probably due to the fact that RTIs are more common in the sexually active reproductive age group. Most of the patients, 197/200 were married. Other authors have also reported a higher rate of RTI in married patients as compared to unmarried patients.[10]

Majority of the patients were multiparous 84.5% (169/200), only 15.5% (31/200) were nulliparous which included three unmarried females. Of the multiparous women, 12/200 had BOH. Another author also reported that incidence of RTI increases with parity. RTI was least common in nulliparous (6%) and highest (76%) in multiparous women.[11]

Candida species were the most common isolate with an isolation rate of 25.5% (51/200) for *Candida albicans* and 5.5% (11/200) for non-albicans candida species. *Neisseria gonorrhoeae* of 0.5% (1/200) was the least common isolate. 11.5% (23/200) samples showed mixed bacterial growth, while 44% (88/200) samples were sterile. The high percentage samples showing no growth in culture were probably due to use of self-medication or use of ointments or lotions by the patient. Bacterial vaginosis was seen in 7% (14/200).

Patnaik and Sahu (2008) has reported Candidiasis in 33.9% of cases, Bacterial vaginosis in 14.3%, *Trichomoniasis* in 12.5% and *N. gonorrhoeae* in 1.8%.[12] Similarly, Madhivanan and Bartman (2009) have reported the prevalence of *T. vaginalis* as 8.5% as compared to 10% in our present study.[13] In another study the incidence of bacterial vaginosis was reported as 20%, Candidiasis as 12.5%, *N. gonorrhoeae* as 1.2% and *T. vaginalis* as 4.1%. These findings are quite different from those of the present study.[14]

The rate of isolation of various organisms from patients with BOH was higher as compared to those with GOH. *Candida albicans* was seen in 33.33% of patients with BOH as compared to 28.03% in GOH, non-albicans *Candida* species in 16.67% of patients with BOH versus 5.09% in GOH. *Gardnerella vaginalis* 8.33% in BOH versus 3.18% in GOH. *Trichomonas vaginalis* was seen in 25% of patients with BOH as compared to 10.83% of patients with GOH.

The differences in isolation rates of organisms in the three groups of patients with RTI viz. multiparous with GOH, multiparous with BOH and nulliparous was found to be highly significant (p value < 0.001). The higher rate of isolation from patients with BOH is probably due to the fact that although these organisms alone are not very important causes of PID, their presence predisposes carriers to acquire other STIs such as HIV and HSV2 virus that have been implicated in causation of endometritis (PID), resultant BOH and infertility.

CONCLUSION

The institution where this study was conducted is located in a small town in eastern Bihar and mainly caters to the rural population of the adjoining areas. This area is part of the Kosi belt, a region known for its poor developmental activities. Consequently, the general population residing here, especially the females are illiterate with poor personal hygiene and occurrence of RTI is a common ailment. In the present study, RTI was seen in 48.5% (97/200) of patients. Vulvovaginal candidiasis was the most common RTI, followed by *trichomoniasis* and bacterial vaginosis. A single case of gonorrhoea was detected.

Studies have shown that common bacterial and fungal agents responsible for RTI do not directly lead to premature

births or recurrent abortions, but their presence predisposes the patients to acquisition of sexually transmitted infections like HSV-2 and HIV, both of which can lead to complications during pregnancy. In our study, the incidence of RTI was higher in the patients with BOH which corroborates the above theory.

Hence, efforts should be made for early diagnosis and treatment of RTIs, as it not only leads to morbidity and distress in patients but it may result in complications during pregnancy, PID and sometimes infertility.

Awareness and sensitisation towards the problem, so that women take proper and complete course of medications rather than reverting to traditional methods of disease control is the need of the hour. New methodologies and interventions should be set up both at service provider level as well as beneficiary end to bring down prevalence rates of RTI.

REFERENCES

[1] Gaash B, Kausar R, Bhan R, et al. Reproductive tract infection in Kargil: a community based study. Indian Journal of Practicing Doctors 2004;1(2):342-4.

[2] Srivastava A, Nandan D, Mehrotra AK, et al. A comparative study of perception about reproductive tract infections among married women in rural, urban and urban slum areas. Indian Journal of Community Medicine 2004;29(2):274-8.

[3] Prasad JH, Abraham S, Kurz KM, et al. Reproductive tract infections among young married women in Tamil Nadu, India. International Family Planning Perspectives 2005;31(2):73-82.

[4] Aljumaili ZKM, Alsamarai AM. Risk factors for bad obstetric history in Kirkuk women, Iraq. Int J Infect Microbiol 2013;2(3):70-7.

[5] Devi R, Srinivas N, Rajangam S. Bad obstetric history and infectious causes. Int J Hum Genet 2002;2(4):269-71.

[6] Agwu AL, Jang SS, Korthuis PT, et al. Pregnancy Incidence and outcomes in vertically and behaviourally HIV-infected youth. JAMA: The Journal of American Medical Association 2011;305(5):468-70.

[7] Winn W, Allen S, Janada W, et al. In: Koneman's Color Atlas and Textbook of diagnostic microbiology. 6th edn. Philadelphia: Lippincott Williams & Wilkins 2006:87-91, 576-95, 1216-20.

[8] Howard AJ, Catherine AI. Haemophilus, Gardnerella and other bacilli. In: Collee JG, Fraser AG, Marmion BP, et al. eds. Mackie and McCartney Practical medical microbiology. 14th edn. New Delhi: Churchill Livingstone 2008: p. 456-8.

[9] Collee JG, Miles RS, Watt B. Tests for Identification of Bacteria. In: Collee JG, Fraser AG, Marmion BP, et al. eds. Mackie and McCartney Practical medical microbiology. 14th edn. New Delhi: Churchill Livingstone 2008: p. 131-49.

[10] Ram R, Bhattacharya K, Goswami DN, et al. Syndromic approach for determination of reproductive tract infections among adolescent girls. Journal of the Indian Medical Association 2006;104(4):178, 180-1.

[11] Rathore M, Vyas L, Bhardwaj AK. Prevalence of reproductive tract infections among women and socio-cultural factors associated with it. Journal of the Indian Medical Association 2007;105(2):71-2, 74, 78.

[12] Patnaik L, Sahu T, Sahani NC. Syndromic diagnosis of RTI/STI among women of reproductive age group. Indian Journal of Community Medicine 2008;4(1):248-54.

[13] Madhivanan P, Bartman MT, Pasutti L, et al. Prevalence of Trichomonas vaginalis infection among young reproductive age women in India: implications for treatment and prevention. Sex health 2009;6(4): 339-44.

[14] Oliviera FA, Pfleger V, Lang K, et al. Sexually transmitted infections, bacterial vaginosis and candidiasis in women of reproductive age in rural Northeast Brazil: a population- based study. Mem Inst Oswaldo Cruz 2007;102(6):751-6.

BACTERIOLOGICAL AND MYCOLOGICAL PROFILE OF BURN WOUND INFECTION IN A TERTIARY CARE HOSPITAL IN KATIHAR, BIHAR

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ABSTRACT

BACKGROUND

Injuries secondary to severe burns rank among the most serious forms of trauma resulting in anatomic, physiologic, endocrinologic and immunologic stress, especially when burns involve more than 20% of Total Body Surface Area (TBSA). In patients surviving the initial burn and resuscitative phase, infections are a leading cause of mortality. The burnt surface is sterile immediately following thermal injury but after 48 hours the wound is colonised with skin pathogens that typically reside in sweat glands and hair follicles before the burn. After 5 to 7 days, wounds become colonised with yeast and/or Gram-positive and Gram-negative organisms from the host's intestinal and upper respiratory tracts, or from the hospital environment and health care workers' hands.

MATERIALS AND METHODS

Surface swabs were collected from 200 patients with burn wound infection of both sexes and all age groups. The microorganisms were identified as per standard protocol based on colony morphology; Gram's staining findings and biochemical tests. ESBL (Extended Spectrum Beta-lactamases) detection was done using PCDDT (Phenotypic Confirmatory Disc Diffusion Test).

RESULTS

A total of 268 organisms were isolated from 200 samples. Out of the 268 isolates, 213 (79.48%) were Gram-negative bacilli, 42 (15.67%) were Gram-positive cocci and 13 (4.85%) were fungi. Among the Gram-negative bacilli, *Pseudomonas aeruginosa* was the most common bacteria isolated at 45.15% (121/268). The isolates exhibited maximum resistance to 3rd generation cephalosporins with good response to piperacillin/tazobactam and carbapenems. Among all the Gram-negative bacilli, 30.99% (66/147) were ESBL producers.

CONCLUSION

The study showed that burn wound infection was more common in females than males. Majority of cases had a burn sustained from an open flame, mostly in winters. Gram-negative bacilli were the predominant isolates (80%) from patients with burn wound infection. Fungi were isolated in about 5% of cases. Pseudomonas aeruginosa was the commonest isolate (45%). Amongst the Gram-positive cocci, staphylococci and enterococci were isolated consistently from patients.

KEYWORDS

Burns, Infections, Gram-Negative Bacilli, Gram-Positive Cocci.

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BACKGROUND

Burn wound infection develops in patients after admission to the hospital. Injuries secondary to severe burns rank among the most serious forms of trauma resulting in anatomic, physiologic, endocrinologic and immunologic stress, especially when burns involve more than 20% of Total Body Surface Area (TBSA).[1]

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After the initial burn and resuscitative phase, infections are a leading cause of mortality. It has been estimated that 75% of all deaths following injuries are due to infections.[2]

The infecting pathogens differ from one hospital to another. The burnt surface is sterile immediately following thermal injury but after 48 hours the wound is colonised with skin pathogens that typically reside in sweat glands and hair follicles before the burn. After 5 to 7 days, wounds become colonised with yeast and/or Gram-positive and Gram-negative organisms from the host's normal intestinal and upper respiratory tracts, or from the hospital environment and health care workers' hands. Fungal infections typically involve the skin or lungs and occur in elderly patients with greater percentage of TBSA and hospital stays longer than 2 to 4 weeks.[1]

Burns are a global public health problem, accounting for an estimated 2,65,000 deaths annually. Non-fatal burns are a leading cause of morbidity, including prolonged

hospitalisation, disfigurement and disability, often with resulting stigma and rejection.

Diagnosis of burn wound infection based on clinical signs and symptoms alone is difficult. Regular sampling of the burn wound either by surface swab or tissue biopsy for culture is done to monitor the presence of infection. Superficial swabs provide an adequate sampling of the microbial flora present on the wound surface and are the most convenient and least invasive approach currently available for excised burn areas.[3]

The present study was taken up with a view to determining the spectrum of microorganisms responsible for causing burn wound infection in our hospital setting and gain knowledge about their antimicrobial susceptibility pattern to help the clinician in deciding on empirical treatment options in cases of severe burn wound infection and imminent septic episodes without having to wait for culture results.

MATERIALS AND METHODS

This prospective study was carried out in the Department of Microbiology, Katihar Medical College, Katihar, Bihar. All patients admitted to the Department of Surgery with burn wound infection were inducted into the study. Institutional ethical committee clearance was obtained before conducting the study. Surface swabs were collected from 200 patients with burn wound infection of both sexes and all age groups. As far as possible, three swabs were collected for microscopy, bacterial and fungal culture. Culture was done on routine bacteriological and fungal culture media.

The microorganisms were identified as per standard protocol based on colony morphology, Gram's staining findings and biochemical tests.[4,5]

All bacterial and yeast isolates were tested against different antimicrobial agents on Mueller-Hinton agar plates by modified Kirby-Bauer disc diffusion technique.[6]

Extended Spectrum Beta-lactamase (ESBL) detection was done using PCDDT (Phenotypic Confirmatory Disc Diffusion Test) with Cefotaxime, Cefotaxime/Clavulanic Acid and Ceftazidime, Ceftazidime/Clavulanic Acid.[6]

Statistical analysis of results was done using the chi square test. P-value ≤ 0.05 was considered to be significant and p- value ≤ 0.001 was considered to be highly significant. All statistical analysis was carried out using online statistical software (http://www.physics.csbju.edu/stats/contingency_NROW_NCOLUMN_form.html; accessed on 18.02.2017).

All media, reagents and antibiotic discs were procured from HiMedia Laboratories, Mumbai.

RESULTS

Out of the total of 200 samples processed in the laboratory, 132 samples showed pure growth of a single organism and 68 showed growths of two organisms.

Table 1 shows the age and sex wise distribution of burn cases. Maximum number of burn cases (69/200) were seen in the age group 21-30 years; out of which 58 (84.06%) were female and 11 (15.94%) were male. Least number of cases (8/200) were seen in the age groups 41 – 50 years; out of which 6 (75%) were female and 2 (25%) were male. The difference in the number of females (58) as compared to the males (11) in the age group 21-30 yrs. was found to be statistically highly significant (p value=0.000).

Table 2 depicts the mechanism of burns. In most of the cases i.e. 88% (176/200), an open flame was responsible for the burns, chemical burns accounted for 1.5% (3/200) of cases. The difference in the number of cases caused by an open flame as compared to other three mechanisms viz. hot liquid, electrical and chemical was found to be statistically highly significant (p value=0.000).

Figure 1 shows the season wise distribution of microorganisms isolated from burn wound cases. Maximum number of cases 40.5% (81/200) was seen in the winter followed by 25% (50/200) of cases seen in the spring season.

Table 3 shows the distribution of microorganisms isolated from burn wound infections. Out of the 268 isolates, 213 (79.48%) were Gram-negative bacilli, 42 (15.67%) were Gram-positive cocci and 13 (4.85%) were fungi. Among the Gram-negative bacilli, *Pseudomonas aeruginosa* was the most common bacteria isolated 45.15% (121/268) and *Citrobacter freundii* was least common 1.49% (4/268). Among the Gram-positive cocci, *Staphylococcus aureus* was the most common isolate 8.21% (22/268) and *Enterococcus faecium* was least common 1.12% (3/268). Among the fungi, *Candida albicans* 2.98% (8/268) was the most common isolate.

Table 4 denotes antimicrobial susceptibility pattern of isolates. Isolates were found to be universally resistant to amoxicillin with significant resistance to 3rd generation cephalosporins [ceftriaxone-100% (92/92), cefuroxime-97.83% (90/92) and cefotaxime-92.86% (198/213) were resistant]. Response to imipenem [84.51% (180/213) were sensitive] and piperacillin/tazobactam [74.65% (159/213) were sensitive] was good among the Gram-negative bacilli. The Gram-positive cocci were found to be universally resistant to cephalexin, significantly resistant to erythromycin 88.10% (37/42). A good number of Gram-positive isolates were found to be sensitive to linezolid 80.95% (34/42) and vancomycin 73.81% (31/42). The enterococci were universally resistant to penicillin, sensitivity to high content gentamicin was 57.14% (4/7) and streptomycin was 42.86% (3/7) respectively whereas sensitivity to teicoplanin was 100% (7/7).

Figure 2 shows the ESBL production among Gram-negative isolates including Pseudomonas species. Maximum ESBL production was noted in *Citrobacter freundii* 50% (2/4) and least in *Pseudomonas aeruginosa* 25.62% (31/121).

Age	Female (%)	Male (%)
0-10	20 (74.07)	07 (25.92)
11-20	21 (55.26)	17 (44.74)
21-30	58 (84.06)*	11 (15.94)*
31-40	15 (45.45)	18 (54.55)
41-50	06 (75.00)	02 (25.00)
51-60	05 (33.33)	10 (66.67)
>60	03 (30.00)	07 (70.00)
Total	**128**	**72**

Table 1. Age and Sex wise Distribution of Burn Cases

*p= 0.000.

Mechanism	No. of Cases	Percentage (%)
Flame*	176	88
Hot liquid	08	04
Electrical	13	6.5
Chemical	03	1.5
Total	**200**	**100**

Table 2. Mechanism of Burns

*p= 0.000.

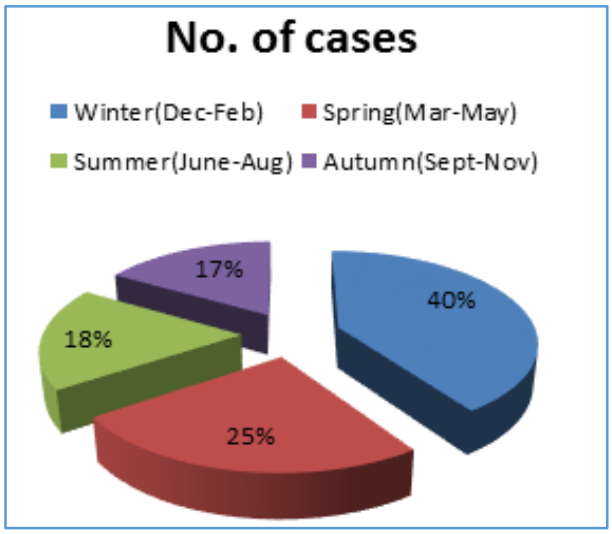

Figure 1. Seasonal Distribution of Burn Cases

Isolate	No.	Percentage (%)
Pseudomonas aeruginosa	121	45.15
Escherichia coli	28	10.45
Proteus mirabilis	25	9.33
Klebsiella pneumoniae	22	8.21
Staphylococcus aureus	22	8.21
Staphylococcus epidermidis	13	4.85
Klebsiella oxytoca	07	2.61
Acinetobacter baumannii	06	2.24
Citrobacter freundii	04	1.49
Enterococcus faecalis	04	1.49
Enterococcus faecium	03	1.12
Candida albicans	08	2.98
Candida tropicalis	05	1.86

Table 3. Microorganisms Isolated from Burn Wound Infections

Antibiotic	*Pseudo. aeruginosa* n=121 (%)	*Esche. coli* n=28 (%)	*Proteus mirabilis* N=25 (%)	*Kleb. pneumoniae* n=22 (%)	*Staph. aureus* n=22 (%)	*Staph. epidermidis* n=13 (%)	*Kleb. oxytoca* n=07 (%)	*Acineto. baumannii* n=06 (%)	*Citrobacter freundii* n=04 (%)	*Entero. faecalis* n=04 (%)	*Entero. faecium* n=03 (%)
CAZ	28 (23.1)										
PI	39 (32.2)										
CIP	24 (19.8)	7 (25.0)	10 (40)	10 (45.5)	10 (45.4%)	3 (23.1)	2 (28.6)	0 (0)	0 (0)	2 (50)%	0 (0)
CPZ	15 (12.4)	1 (3.6)	1 (4)	0 (0)			0 (0)	0 (0)	0 (0)		
CTX	15 (12.4)	0 (0)	0 (0)	0 (0)			0 (0)	0 (0)	0 (0)		
CPM	14 (11.6)	0 (0)	4 (16)	1 (4.5)			2 (28.6)	0 (0)	0 (0)		
AK	49 (40.5)	12 (42.9%)	7 (28)	2 (9.1)	14 (63.6%)	5 (23.07)	5 (71.4)	0 (0)	0 (0)		
GEN	21 (17.3)	6 (21.4)	2 (8)	5 (22.7)	8 (36.4)	0 (0)	0 (0)	0 (0)	0 (0)		
TOB	29 (24.0)										
PIT	100 (82.6%)	21 (75.0)	22 (88)	9 (40.9)			5 (71.4)	0 (0)	2 (50)		
CFS	18 (14.9)	2 (7.1)	2 (8)	1 (4.5)			5 (71.4)	0 (0)	0 (0)		
IPM	118 (97.5%)	17 (60.7%)	22 (88)	9 (40.9)			7 (100)	5 (83.3)	2 (50)		
CXM		0 (0)	2 (8)	0 (0)			0 (0)	0 (0)	0 (0)		
CTR		0 (0)	0 (0)	0 (0)			0 (0)	0 (0)	0 (0)		
CN					0 (0)	0 (0)					
E					3 (13.6)	0 (0)				1 (25%)	1 (33.3%)
NET					21 (95.4%)	11 (84.6)					
VA					19 (86.4%)	7 (31.8)				2 (50%)	3 (100%)
LZ					19 (86.4%)	11 (84.6)				2 (50)%	2 (66.6%)
CD					7 (31.8)	5 (38.4)					
TEI										4 (100%)	3 (100%)
TE										2 (50%)	1 (33.3%)
HLG										3 (75%)	1 (33.3%)

Table 4. Antibiotic Susceptibility Pattern of Isolates

CAZ- Ceftazidime; PI- Piperacillin; CIP- Ciprofloxacin; CPZ- Cefoperazone; CTX- Cefotaxime; CPM- Cefepime; AK- Amikacin; GEN- Gentamicin; TOB- Tobramycin; PIT- Piperacillin/Tazobactam; CFS- Cefoperazone/ Sulbactam; IPM- Imipenem; CXM- Cefuroxime; CTR- Ceftriaxone; CN- Cephalexin; E- Erythromycin; NET- Netilmicin; VA- Vancomycin; LZ- Linezolid; CD- Clindamycin; TEI- Teicoplanin; TE- Tetracycline; HLG- High level Gentamicin.

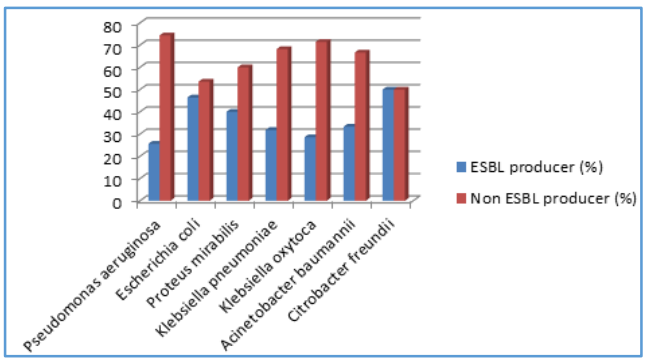

Figure 2. ESBL Production among Gram-negative Isolates

DISCUSSION

Majority of patients (69/200) in this study were in the age group 21-30 yrs, out of which 84.06% were female. This was followed by the age group 11-20 yrs. (38/200) among which 55.26% were female and 44.74% were male. Similar findings have been reported by other authors.[7,8,9] A few authors on the other hand have reported more number of males.[10,11,12]

The preponderance of female patients may be due to the fact that they tend to spend more time in the kitchen cooking over open flames.

In most cases, the cause of burn production was an open flame - 88%. The electric burns accounted for 6.5% followed by scalding (in hot liquid) 4% and chemical burns 1.5%. Other authors have also reported maximum number of burns due to flame injury (66.0%) followed by scalding (16%).[10] Likewise another group of authors have reported 87.81% of flame burns, followed by scalds 10.08% and electrical burn 2.11%.[7] The findings of these studies are more or less similar to the findings of the present study except that in our study, the number of electrical burns was more than the number of burns caused by scalding.

Majority of burn cases were seen in winter (40.50%). The cases correspondingly reduced to 25% in spring, 18% in summer and 16.50% in autumn. The greater number of cases in winter can be explained by the fact that people, especially in rural areas have a habit of sitting around open flames for warmth.

Pseudomonas aeruginosa was the most common isolate in cases of burn wound infection 45.15%, followed by *Escherichia coli* 10.45%, *Proteus mirabilis* 9.33% and *Staphylococcus aureus* and *Klebsiella pneumonia* 8.21% each. *Candida albicans* accounted for 2.98% and *Candida tropicalis* for 1.86% of isolation. (Table 3)

Similar reports were made by other authors with *Pseudomonas aeruginosa* (30%) being the most common isolate followed by *Staphylococcus aureus* (28%) and Klebsiella species (16%).[13] A few others have, however, reported *Staphylococcus epidermidis* (56.63%) as the most common isolate followed by *Pseudomonas aeruginosa* (18.18%) and *Staphylococcus aureus* (13.63%).[14] A retrospective study of isolations from burn wound infection from 1997 to 2002 and from 2002 to 2005, however, reported that the number of Pseudomonas infections reduced from 58.95% in the 1st study to 51.5% in the 2nd study. There was increase in the number of Acinetobacter infections from 7.22% to 14.23%.[2] The incidence of *Acinetobacter baumannii* infection in our study was found to be 2.24%.

In another study, Staphylococcus was the most common isolate 47.8%, followed by *Pseudomonas aeruginosa* 23.0% and *Candida albicans* and *Escherichia coli* 5.3% each.[11] The variations seen in the isolation pattern of different organisms in all these studies could be due to the geographical locations in which these studies were conducted and the differences in hospital microenvironment in different hospitals. However, *Pseudomonas aeruginosa* appears to be a major pathogen in all these studies as seen in the present study.

Gram-negative bacilli showed maximum resistance to amoxicillin and 3rd generation cephalosporins. Least resistance was seen with imipenem and piperacillin/tazobactam. (Table 4).

The high degree of resistance exhibited by these isolates was probably due the fact that they were hospital-acquired strains. Another reason could be the gross misuse of antibiotics in hospital settings especially cephalosporin group of antibiotics.

Other authors have also reported 100% resistance to ampicillin in *Escherichia coli* and Proteus species which is quite similar to the findings of present study where all Gram-negative isolates including *Escherichia coli* and Proteus species showed 100% resistance to amoxicillin.[13] Few authors have reported 100% sensitivity to imipenem which is bit different from the findings of present study where 15.49% of the strains were found to be resistant to imipenem.[2] Another study reported that 93.34% strains of Klebsiella species were resistant to ceftriaxone which is again similar to the findings of the present study where 100% resistance was seen with ceftriaxone, 97.83% with cefuroxime and 92.86% with cefotaxime.[8]

42.86% of Staphylococci were methicillin resistant and 23.81% were vancomycin resistant as detected by disc diffusion test. Maximum resistance was seen with Amoxicillin and Cephalexin (100%). Least resistance was seen with Netilmicin (8.57%) and Linezolid (19.05%).

Some authors have reported 35.6% of MRSA strains and others 40.0% methicillin-resistant strains in Staphylococci which is similar to the findings of the present study. Vancomycin resistance was, however, not encountered in these studies.[9,8]

Among all the Gram-negative bacilli, 30.99% were ESBL producers. Maximum ESBL production was seen in *Citrobacter freundii* in which 50% of the strains were ESBL producers. However, as the number of Citrobacter isolated were very few (only 4 isolates), this finding is probably not a true representation of ESBL production in this species. ESBL production was seen in 46.43% of *Escherichia coli*, 40.00% of *Proteus mirabilis* and 31.82% of *Klebsiella pneumoniae*, 28.57% of *Klebsiella oxytoca*, 25.62% of *Pseudomonas aeruginosa* (Figure 2).

Others have found 42.9% of Klebsiella spp. to be ESBL producers, followed by *Escherichia coli* 25.0% and Proteus spp. 21.4%. Overall 21.4% of their isolates were ESBL producers.[15] The findings of this study are different from those of the present study.

Another group of authors found ESBL production in 39.8% of Gram-negative bacilli as compared to 30.99% in our study. In their study, however, *Pseudomonas aeruginosa* was the predominant ESBL producer 20.5% followed by *Klebsiella pneumoniae* 7.2%.[16]

CONCLUSION

In the present study, it was seen that burn wound infection was more common in females than males. Majority of cases had burn sustained from an open flame with most cases occurring in winter. As seen in all types of health care associated infections, Gram-negative bacilli were the predominant isolates from patients with burn wound infection accounting for 80% of cases. This is because Gram-negative bacilli are hardier and adapt better to hospital environment than Gram-positive cocci, with a few exceptions like staphylococci and enterococci. Fungi were isolated in about 5% of cases indicating that they may play a greater role in burn wound infection especially in this era of widespread use of antibiotics. As expected Pseudomonas aeruginosa was the commonest isolate accounting for 45% of total isolations. Amongst the Gram-positive cocci, it was seen that the hardier ones like Staphylococci and Enterococci were isolated consistently from patients with burn wound infection.

Infection control measures like strictly enforced hand washing and universal use of personal protective equipment like gowns, gloves and mask needs to be strictly adhered to. Practices like early excision therapy can be performed as a bedside procedure to prevent cross-infection. Role of infection control practices, regular surveillance cultures especially for detection of MRSA, VRE, ESBL producing Gram-negative bacilli also need to be highlighted. Routine cultures from burn wounds and other sources like blood, respiratory and urine samples should be monitored to identify epidemic pathogens and antibiotic resistant strains so that infection control measures can be immediately implemented.

REFERENCES

[1] Murray CK. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. 7th edn. Philadelphia: Churchill Livingstone 2010:3905-8.

[2] Mehta M, Dutta P, Gupta V. Bacterial isolates from burn wound infections and their antibiograms: a eight year study. Indian Journal of Plastic Surgery 2007;40(1):25-8.

[3] Church D, Elsayed S, Reid O, et al. Burn wound infections. Clin Microbiol Rev 2006;19(2):403-32.

[4] Collee JG, Miles RS, Watt B. Mackie and McCartney practical medical microbiology. 14th edn. New Delhi: Churchill Livingstone, 2008:131-49.

[5] Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's diagnostic microbiology. 12th edn. Missouri: Mosby Elsevier, 2007:120-46.

[6] CLSI. Performance standard for antimicrobial sensitivity testing: eighteenth informational supplement. M100-S18, 2008;28(1):162-3.

[7] Akhter JM, Nerker NE, Reddy PS, et al. Epidemiology of burned patient admitted in burn unit of a tertiary teaching hospital. Pravara Med Rev 2010;2(4):11-7.

[8] Shahzad MN, Ahmed N, Khan IH, et al. Bacterial profile of burn wound infections in burn patients. Ann Pak Inst Med Sci 2012;8(1):54-7.

[9] Idomir M, Pirau R, Nemet C, et al. Evaluation of microbiological spectrum of burn wound infections. Bulletin of the Transilvania University of Brasov Series VI: Medical Sciences 2012;5(54):7-12.

[10] Chalise PR, Shrestha S, Sherpa K, et al. Epidemiological and bacteriological profile of burn patients at Nepal medical college teaching hospital. Nepal Med Coll J 2008;10(4):233-7.

[11] Alghalibi SM, Humaid AA, Alshaibani EA, et al. Microorganisms associated with burn wound infection in Sana'a, Yemen. Egypt Acad J Biol Sci 2011;3(1): 19-25.

[12] Al-Ibran E, Meraj S, Nasim M, et al. Pattern of microorganisms isolated from flame burn wounds and their trends of susceptibility to antibiotic during past three years. Journal of the Dow University of Health Sciences 2013;7(2):49-53.

[13] Shaikh D, Zaidi SAH, Shaikh K, et al. Incidence and resistance pattern of bacteria associated with burn wound sepsis. Pakistan Journal of Pharmacology 2004;21(2):39-47.

[14] Dhar S, Saraf R, Singh K, et al. Microbiological profile of chronic burn wounds among patients admitted in burn unit. JK Science 2007;9(4):182-5.

[15] Islam MS, Yusuf MA, Chowdhury MSJH, et al. ESBL producing gram negative aerobic bacteria isolated from burn wound infection with their antibiogram in Dhaka. Journal of Science Foundation 2012;10(2): 63-9.

[16] Bandekar N, Vinodkumar CS, Basavarajappa KG, et al. Beta lactamases mediated resistance amongst Gram-negative bacilli in burn infection. International Journal of Biological & Medical Research 2011;2(3):766-70.