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PREVALENCE OF ENTEROCCAL INFECTIONS AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN FROM A TERTIARY CARE INSTITUTE

Microbiology

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ABSTRACT

Enterococci form an indigenous flora of the intestinal tract, oral cavity and the genitourinary tract of humans and animals. Once regarded as human commensals have emerged as significant nosocomial pathogens. They have high mortality rates, show intrinsic resistance to many antimicrobial agents, capable of transferring resistance genes to other bacteria hence emerge as leading clinical challenges for physicians. 225 Enterococcal isolates were obtained from various clinical samples. They were further differentiated to species level of which 203 (90.22%) isolates were identified as E.faecalis, 12 (5.33%) as E.faecium and 10 (4.44%) as E.durans. Isolates showed good sensitivity to Linezolid(100%) and Vancomycin(99.11%), E.faecalis(45.81%) and E. faecium(66.67%) had decreased sensitivity to Gentamicin. Treatment with combination therapy of aminoglycoside with a cell wall active agents has been recommended. Detection of resistance to aminoglycosides is of utmost important to prevent failure of combination therapy. Management of these infections with appropriate antibiotics yield desirable results.

KEYWORDS

Enterococcus, nosocomial pathogen, Antimicrobial Resistance.

Introduction :

Enterococci are gram positive facultative anaerobic organisms occurring singly, in pairs or in short chains. Enterococci form an indigenous flora of the intestinal tract, oral cavity and the genitourinary tract of humans and animals[1]. Several species of Enterococcus have been identified till date of which two species Enterococcus faecalis and Enterococcus faecium are most common cause of infections in humans. Enterococci once regarded as human commensals have emerged as significant nosocomial pathogens[1,2]. A study from the Centre for Disease Control National Nosocomial Surveillance lists Enterococci as the most common cause of nosocomial urinary tract infections[1]. The reasons for Enterococci rising as a nosocomial pathogen is due to excessive use of antineoplastic, biological and other immunosuppressive agents[3]. They are formidable pathogens having high mortality rates because of their resistance to antimicrobial agents thus allowing them to survive and thrive in hospital environment. They are intrinsically resistant to many antimicrobial agents, they readily acquire resistance genes and are capable of transferring resistance genes to other bacteria like Staphylococcus aureus, these characteristics are largely responsible for their success as pathogens despite their limited virulence properties. Enterococcal infections, hence have emerged as one of the leading clinical challenges for physicians when identified as a cause of serious life threatening infections like meningitis or endocarditis [2].They act as opportunistic pathogens and are gaining importance as causative agents for invasive and non invasive nosocomial infections in severely ill and immune compromised patients[4].

Therapeutic short coming of Enterococci was largely overcome by adding an aminoglycoside to Penicillin or another cell wall synthesis inhibitor, such as Vancomycin, but longer duration of therapy and increased toxicity of combination regimens marked these cocci problematic with respect to antimicrobial therapy. The synergistic bactericidal effects between aminoglycosides and beta lactums or glycopeptide antibiotics (cell wall active agents) is lost if there is high level resistance to either class of drug. Resistance to high concentration of aminoglycosides usually mediated by aminoglycoside modifying enzymes which are wide spread among Enterococci[5,6]. Increase in resistance to Penicillin and Ampicillin has been observed in recent years in Enterococcus faecium than Enterococcus faecalis, due to this Vancomycin is rapidly becoming drug of choice for treatment of infections caused by multi drug resistant Enterococci[2]. Uttley et al were first to report Vancomycin resistance in Enterococci from England in 1988[7]. The scenario since then has worsened with data from CDC and Prevention in USA shows incidence of Vancomycin resistant Enterococci increased many a fold[6].

Characterization of patients involved in outbreak situations, have identified several predisposing factors for serious Enterococcal infections which include severe underlying disease, hospitalization for an extended period, prior multiple antibiotic treatment and immune compromised status to name a few. Wide spread resistance to multiple antibiotics is also forcing clinicians to experimental antimicrobial agents and combinations whose effectiveness is not yet proven[2,4].

Present study was undertaken to determine

1. Incidence of Enterococcal isolation from various clinical samples.
2. Antimicrobial susceptibility pattern of Enterococci isolated from various clinical samples.

Materials and Methods :

The present study was carried out in a tertiary care hospital over a period of two years. Enterococcal isolates obtained from all specimens during the period were included. The culture media used for primary isolation of pathogenic bacteria were Blood agar, MacConkey's agar and Chocolate agar. The isolates were identified to species level as per standard protocol[8,9]. Enterococci were speciated based on colony morphology, grams staining, catalase test, bile esculin hydrolysis and growth in 6.5% sodium chloride. Antimicrobial susceptibility testing of all isolates were done by Kirby Bauer Disk Diffusion Method (KBDDM) as per CLSI guidelines[10] for the following antibiotics Penicillin, Ampicillin, Vancomycin, Teicoplannin, Linezolid, Erythromycin, Tetracycline, Rifampicin, Chloramphenicol, Gentamicin, Ciprofloxacin, Nitrofurantoin.

Results :

The study was performed over a period of two years in a multi-specialty tertiary care hospital. The total number of samples received for culture were 21524 of which 9600 included pus and wound discharge, 5164 urine samples, 4798 samples of blood culture and 1962 samples of high vaginal swabs. Amongst 9600 pus and wound discharge for culture 5980 showed growth in culture and of these 125 samples grew Enterococci species. Of the 5164 urine samples received for culture, 1601 showed growth and 54 samples grew Enterococci species. From 4798 blood cultures processed 609 showed growth in culture and 32 samples yielded Enterococci species. 1962 high vaginal swabs processed for culture 589 had growth in culture of which 14 samples grew Enterococci.

In a period of two years 225(2.56%) Enterococci species were isolated from various clinical samples, pus samples yielded 125 Enterococci species - 55.55% , 54 Enterococci species from urine - 24%, 32 Enterococci species isolated from blood cultures - 14.22% and 14 from high vaginal swabs - 6.22%.The overall rate of infection of Enterococci was 2.56% of these 69 (30.66%) isolates were further identified as colonizers i.e. 34 isolates from urine, 23 from pus and 12

from high vaginal swabs were colonizers and 156 (69.33%) isolates were actual pathogens hence actual rate of infection by Enterococci is 1.78%.

Two hundred and twenty five isolates were further differentiated to species level of which 203 (90.22%) were identified as E.faecalis, 12 (5.33%) as E.faecium and 10 (4.44%) as E.durans.

Antibiotic susceptibility testing for all isolates was done by KBDDM for the following antibiotics – Penicillin, Ampicillin, Vancomycin, Linezolid, Teicoplanin, Rifampicin, Gentamicin, Ciprofloxacin, Tetracycine, Chloramphenicol, Erythromycin and Nitrofurantoin. Erythromycin was not tested for urinary isolates and Nitrofurantoin was tested only for urinary isolates. All E.faecalis strains were sensitive to Linezolid and Teicoplannin - 100%, sensitivity to Vancomycin was 99.5%. Sensitivity to Ampicillin and Penicillin was 79.31% and 69.45% respectively. 45.81% of the isolates were sensitive to Gentamicin and 30.45% to Ciprofloxacin. In case of E.faecium all strains were sensitive to Linezolid but one strain was resistant to Vancomycin and Teicoplannin. Sensitivity to Gentamicin and Ciprofloxacin was 66.67% and 16.67% respectively. All strains of E.durans were sensitive to most of the antibiotics tested.

Table No. I: Isolation Of Enterococci From Clinical Samples

Sr. No.	Kind of Samples	Number Received	Culture Positivity	Enterococci Positive Samples Number / Percent
1.	Pus and Wound Discharge	9600	5980	125 (55.55%)
2.	Urine	5164	609	54 (24.00%)
3.	Blood Cultures	4798	1601	32 (14.22%)
4.	High Vaginal Swabs	1962	589	14 (6.22%)

Table No. II : Susceptibility Pattern Of Enterococci

Sr No	Antibiotics Tested	Disc Content	E.faecalis Sensitivity %	E.faecium Sensitivity %	E.durans Sensitivity %
1.	Penicillin	10 units	69.45	66.67	80.00
2.	Ampicillin	30 mcg	79.31	66.67	100
3.	Vancomycin	30 mcg	99.50	91.67	100
4.	Linezolid	30 mcg	100	100	100
5.	Teicoplannin	30 mcg	100	91.67	100
6.	Gentamicin	120 mcg	45.81	66.67	100
7.	Ciprofloxacin	05 mcg	30.54	16.67	100
8.	Rifampicin	05 mcg	95.56	66.67	80.00
9.	Tetracycline	30 mcg	38.42	50.00	80.00
10.	Chloramphenicol	30 mcg	74.88	33.33	70.00
11.	Erythromycin * n=171	15 mcg	16.37	36.36	71.43
12.	Nitrofurantoin ** n=54	300 mcg	51.85	100	100

*Excludes urinary isolates **Only urinary isolates

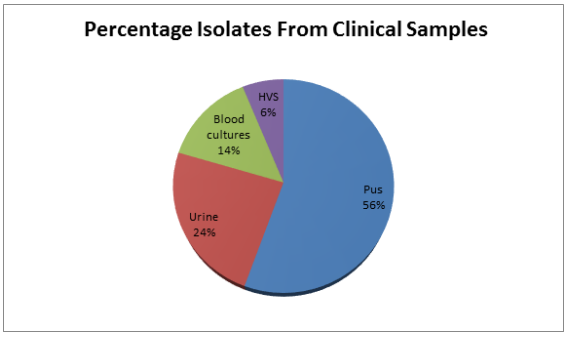

Figure 1: Percentage Isolation of Enterococci from Clinical Samples

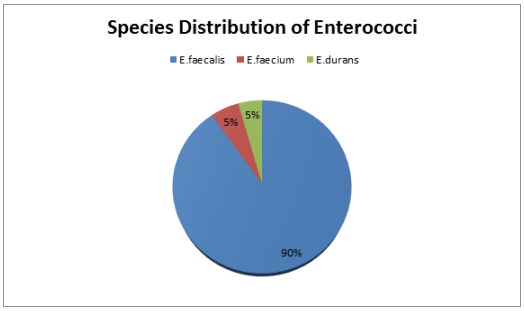

Figure 2: Speciation Of Enterococci

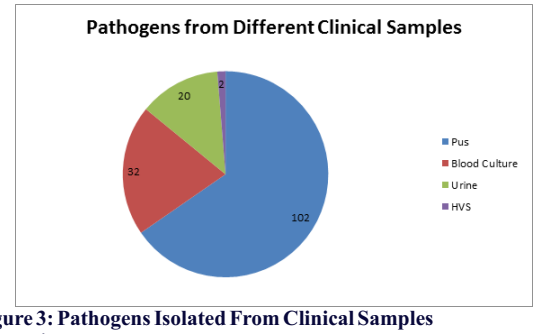

Figure 3: Pathogens Isolated From Clinical Samples

Discussion :

Enterococci which is an indigenous flora of the oral cavity, intestinal tract and genitourinary tract of humans are relatively avirulent in healthy individuals[1,11]. These organisms have emerged as nosocomial pathogens of significant concern in the last few decades due to changes in hospital patient population and imprudent use of antimicrobials[11,12]. They do not cause severe systemic inflammatory response but present as therapeutic challenge due to resistance to commonly used antimicrobials such as Gentamicin, Penicillin, Ampicillin and sometimes Vancomycin. The emergence of Vancomycin Resistant Enterococci is a major concern as there are limited therapeutic options for treating life threatening infections such as endocarditis, meningitis and the potential of these organisms to transfer Vancomycin resistance genes to other gram positive cocci such as Methicillin Resistant Staphylococcus aureus.

In the present study 225 Enterococci were isolated from 21524 clinical samples over a period of two years. The overall prevalence of Enterococci from various sites was 2.56%. Majority of the isolates were from pus and wound discharge i.e. 55.55% followed by urinary tract 24.00% and then blood cultures 14.22%. The site distribution is comparable to the NNIS studies except the overall incidence of Enterococci is 10% of which 17% were from urinary tract 12% from surgical sites and 9% from blood which is slightly higher than our study[13]. Gordon et al and McNamara et al have reported isolation of Enterococci from urinary tract followed by wound and soft tissues[14,15]. Enterococci form a major part of endogenous flora of the intestinal tract, oral cavity and genitourinary tract. 69.33% (156) of isolates from the study were categorized as pathogens as they were grown from pus, blood cultures and urine. The clinical history and correlation with infection was considered and the isolates were labeled as pathogens. Most of the isolates were obtained from pus and urine whose hospital stay was for a longer duration which is in agreement with Desai et al[11]. 30.66% (69) isolates were labeled as colonizers by correlation with clinical history and examination of the site in wounds where as presence of pus cells and organisms in urine in catheterized patients was an indication for pathogens.

The 225 strains were further identified as E.faecalis 203(90.22%)E.faecium 12(5.33%) and E.durans 10(4.44%) which can be compared with various studies in India. Bhatt et al reported incidence of E.faecalis 86.9%, E.faecium 10.7%, E.raffinoses 6% and E.hirae 1%[16] Agrawal et al reported the incidence of species 86% E.faecalis and 14% E.faecium[17]. Miskeen et al reported 87.07% E.faecalis 10.88% E.faecium and 2.05% E.durans[18]. International studies have reported similar results Gordon et al recorded E.faecalis as 90%, E.faecium as 8% and other enterococci species as 2%[14]. Hallgren et al have reported 76% of E.faecalis followed by 23% of E.faecium and 1% of other enterococci species in their study[19]. A Polish study states E.faecalis and E.faecium as the two common species from

most of the clinical infections[4]. From the present study and many other studies from India and abroad it is evident that E.faecalis and E.faecium are the two common species causing infections in human beings. Other Enterococcal species which include E.avium, E.raffinoses, E.hirae, E.durans, E.casseliflavus and E.malodaratus are infrequently isolated from human infections.

The Penicillin resistance in the study is 30.22%, Miskeen et al have reported it as 23.3%, this low level of resistance to Penicillin is an intrinsic property of Enterococci which can be beneficial as a synergistic Penicillin aminoglycoside combination therapy [18] 99.11% of the strains were sensitive to Vancomycin which is in accordance with a study by Mathur et al where 444 isolates of Enterococci were studied and resistance in Vancomycin was as 1% reported by disk diffusion method[20]. In another study by Miskeen et al no strain was resistant toVancomycin[18]. A Japenese study by Kajihara et al on Enterococci recorded no resistance to Vancomycin in their isolates[3]. The Vancomycin Resistant Enterococci (VRE) rates in China are 6% in E.faecalis and 2.7% in E.faecium isolates respectively thus the detection rate of VRE in Asia is low[21,22]. Vancomycin resistance is relatively higher in developed countries as shown by Pegues et al who reported 82% and Boyle et al report 92.3% resistance to Vancomycin in Enterococci which can be attributed to greater use of Vancomycin in developed countries[23,24]. Low level of Vancomycin resistance in our country and other developing nations in Asia is due to economic reasons thus not using the drug indiscriminately and saving one drug of much importance for many fatal infections. The overall resistance to Gentamicin was 50.67% of which 54.11% E.faecalis strains and 33.33% E.faecium were resistant to Gentamicin. High level of Gentamicin resistance has been reported from most countries, prevalence ranging from 1 - 48%.Patterson et al reported 27.6% resistance and Miskeen et al reported 36.7% resistance in Enterococci to Gentamicin[20,25]. A high level of Gentamicin resistance was also seen in Poland by Gawryszewska et al where 50% of strains were resistant to Gentamicin, such high level Gentamicin resistance excludes the possible use of combination therapy of aminoglycosides with penicillins & glycopeptides (cell wall active agents) for an enhanced bactericidal effect[4]. Treatment of these infections with appropriate antimicrobials post antibiotic susceptibility testing is need of the hour which will help clinicians with timely management and limit the spread of antimicrobial resistance.

Summary & Conclusions :

Treatment of serious Enterococcal infections with combination therapy of aminoglycoside with a cell wall active agent such as penicillins or glycopeptides has been recommended. Distinguishing high level resistance strains to aminoglycosides from intrinsic resistant strains is of vital importance. Therefore it is recommended that all isolates should be tested for high level aminoglycoside resistance, this would help to limit intrahospital dissemination of resistance and help to establish a surveillance programme about the use of Vancomycin and aminoglycosides for management of Enterococcal infections.

Enterococci have emerged as a major nosocomial pathogen with high level Gentamicin resistance. Treatment of these infections with appropriate antibiotics and judicous use of reserve drugs like Vancomycin and Linezolid would help in management of such life threatening infections and have a desirable outcome thus bringing relief to the treating physician in the era of rising antimicrobial resistance.

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