National Journal of Medical and Allied Sciences

[ISSN Online: 2319 – 6335, Print: 2393 – 9192|Original article |Open Access]

Website:-www.njmsonline.org

A RETROSPECTIVE ANTIBIOGRAM STUDY OF CLINICAL ISOLATES OF ENTEROCOCCUS AMONG PATIENTS ATTENDING AT THE IIMS&R HOSPITAL, LUCKNOW

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ABSTRACT

Background: Enterococci are gram-positive cocci, spectacle-shaped in appearance. They are considered relatively low virulence, but now, they are becoming critical nosocomial pathogens. They are causing various clinical manifestations like UTI, endocarditis, intra-abdominal and pelvic infections. They pose a severe threat to mankind with their ability to resist multiple drugs, with some isolates being resistant to almost all the antibiotics tested.

Materials and Methods: Two hundred clinical isolates were collected from Integral Institute of Medical Sciences and Research, Lucknow. Various samples were collected, such as urine, pus, blood, vaginal swab on the basis of clinical symptoms. Antimicrobial susceptibility pattern was performed by Modified Kirby Bauer disk diffusion method. The antibiotics tested were Penicillin, Ampicillin, Vancomycin, Linezolid, Teicoplanin, Doxycycline, Tetracycline, Ciprofloxacin, Norfloxacin, Nitrofurantoin, Erythromycin, High-level Gentamicin, High-level Streptomycin.

Results: Out of 200 clinical isolates processed, 40(80%) were from urine, 3(6%) were from pus,5(10%) were from blood,2(4%) were from a vaginal swab. Ampicillin shows the highest resistance (92%), followed by Penicillin (88%), among which 6% Enterococci were Vancomycin-resistant.

Conclusion: This study demonstrates the antimicrobial susceptibility pattern of Enterococcal isolates, with some isolates are resistant to almost all antibiotics tested, posing a severe therapeutic challenge to mankind.

Keywords: Nosocomial pathogens, Virulence factors, Prevalence, Antibiotic resistance, Antibiotic sensitivity, VRE

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INTRODUCTION

Enterococci are gram positive, non-motile cocci(except E. gallinarum, E. casseliflavus) belonging to the family Enterococcaceae and are arranged in angulated pairs(spectacle shaped). They are the normal flora of the human gastrointestinal tract and are also critical nosocomial pathogens.[1] Enterococcus have been considered as relatively low virulence[2]. Still, several reports have registered that the two most important species (E.faecium, E.faecalis) are the leading causes of opportunistic human infections[3], including UTI[4], surgical sites infections, burn wound infections[5,6], bacteremia and sepsis[7], endocarditis[8], cholecystitis[9], peritonitis[10], neonatal meningitis[11], and others. Virulence factors such as Enterococcal surface protein(ESP), aggregation substances(pheromones), capsule formation and gelatinase are involved in bacterial adherence to host cells and biofilm formation on surfaces in hospital environment[12-16]. The increasing evidence of healthcare-associated Enterococcal infections is mainly the result of bacterial features such as expression and transfer of genetic material, increasing their antimicrobial resistance and pathogenecity[17-19]. The severity of Enterococcal infections has increased due to its ability to resist antimicrobial drugs. Resistance can be of two types, it can be intrinsic such as resistance to low level of

aminoglycosides, cephalosporins and penicillin or can be acquired such as resistance to glycopeptides, e.g. vancomycin and teicoplanin[20].VRE infections are life-threatening and lead to higher mortality rates because glycopeptides are considered the last treatment available[21].VRE is mediated by a group of genes(van A, van B, van C, van D and van E)[21].

National Health Safety Network summary report, between 2009 and 2010, reported that Enterococci were the second common cause of nosocomial infections. The report showed that Enterococci were 14%, next to S. Aureus (16%), and among these,3% Enterococci were Vancomycin resistant[22]. VRE reported in Europe (4%), Asia Pacific (11.9%), America(35.5%) and Latin America(12.9%)[23].

With the above background, this study was undertaken to determine the prevalence of Enterococcus along with its antibiogram isolated from various samples in patients attending IIMS & R, Lucknow.

MATERIAL AND METHODS

This retrospective study was conducted at Integral Institute of Medical Science and Research, Hospital, Lucknow, from April 2018 to March 2019. The study was approved by the Institutional Research Committee (IRC) and the Ethical Research Committee (ERC). Samples such as pus, vaginal swabs, blood, urine delivered to the microbiology laboratory for culture and sensitivity were processed from both IPD and OPD. Direct smear microscopy of several clinical specimens was performed. Gram-positive cocci (spectacle-shaped) was seen. Then, specimens were inoculated onto Blood agar, MacConkey agar, Cystine Lactose Electrolyte-Deficient (CLED) agar, and incubated at 37°C for 24 hours. The culture plates were examined the relative numbers and types of colonies were noted and processed further. Blood agar produces non-hemolytic translucent colonies(Gamma type of hemolysis). MacConkey agar produces minute magenta pink colonies. CLED agar produces lactose fermenting colonies.

Confirmation of Enterococcus- Enterococcus growth was confirmed by biochemical tests such as the Bile aesculin hydrolysis test.

BILE AESCULIN HYDROLYSIS TEST- Enterococcus gives a positive bile aesculin hydrolysis test (they grow in the presence of 40% bile and hydrolyses aesculin into aesculetin that combines with ferric chloride to produce black coloured complex).

Antimicrobial Susceptibility Testing- Antimicrobial susceptibility test of all isolates was performed on Mueller Hinton agar by disk diffusion method (Modified Kirby Bauer disk diffusion method). The result was interpreted according to CLSI guidelines 2018 [24].

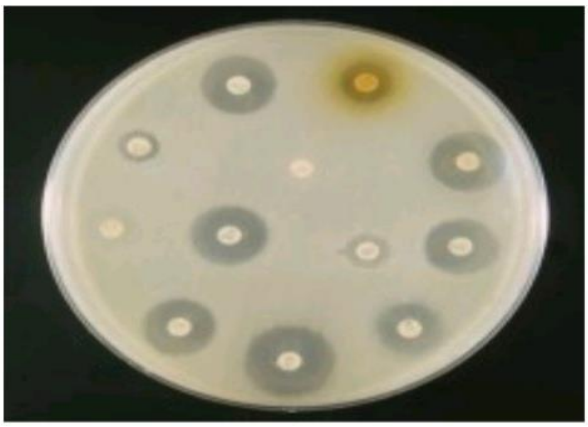

Figure: Mueller Hinton Agar (MHA) plate used for antibiotic sensitive test

Statistical Analysis**:** Data was analyzed using Ms Excel and SPSS 16 version.

RESULTS

During the study period, 200 clinical isolates were collected from patients admitted from the Integral Institute of Medical Science and Research (IIMS&R), Lucknow. Various specimens like urine, blood, pus, vaginal swab were processed. Out of which 50 enterococcal isolates, 40(80%) were from urine, 3(6%) were from blood, 5(10%) were from pus, and 2(4%) were from a vaginal swab.

Table 1: Prevalence of Enterococcus among Clinical Specimen

Samples	Total Samples	Positive Samples	% of Positive Samples
Urine	50	40	80%
Pus	50	3	6%
Blood	50	5	10%
Vaginal Swab	50	2	4%
Total	200	50	100%

Out of 50 Enterococcal isolates included in this study, 30(60%) were isolated from IPD and

20(40%) were isolated from OPD patients. Out of 50 Enterococcal isolates in the study, 35(70%) were females, and 15(30%) were male patients.

Table 2: Distribution of patients according to age groups

Age Group (yrs)	No. of Patients	% of Patients
0-10	3	6%
11-20	6	12%
21-30	12	24%
31-40	16	32%
41-50	6	12%
51-60	4	8%
61-70	3	6%
TOTAL	50	100%

Out of 50 Enterococcal isolates in the study, the maximum number of patients belonged to age group 31-40 (16), followed by age group 21-30(12), followed by age group 41-50 and 11-20(6), followed by age group 51-60(4), followed by age group 61-70 and 0-10(3). (Table 2)

Table 3: Antimicrobial Resistance Pattern of Enterococcus Isolates

Antibiotics Used	Isolates Resistance %
AMPICILLIN(AMP)	92%
PENICILLIN(P)	88%
CIPROFLOXACIN(CIP)	74%
DOXYCYCLINE(DO)	62%
HLG	60%
NORFLOXACIN(NX)	54%
ERYTHROMYCIN(E)	54%
HLS	42%
NITROFURANTOIN(NIT)	12%
TEICOPLANIN(TEI)	10%
TETRACYCLINE(TE)	10%
VANCOMYCIN(VA)	6%
LINEZOLID(LZ)	2%

Table 3 shows the resistance pattern of Enterococcus. Ampicillin (AMP) shows the highest resistance (92%), followed by Penicillin(P) 88%, followed by Ciprofloxacin(CIP) 74%, followed by Doxycycline (DO) 62%, followed by HLG 60%, followed by Norfloxacin (NX) 54%, followed by Erythromycin (E) 54%, followed by HLS 42%, followed by Nitrofurantoin (NIT) 12%, followed by Teicoplanin (TEI) 10%, followed by Tetracycline (TE) 10%, followed by Vancomycin (VA) 6% and Linezolid (LZ) 2%.

Table 4: Antimicrobial Sensitivity Pattern Of Enterococcus

Antibiotics Used	Isolates Sensitive (%)
VANCOMYCIN(VA)	94%
TEICOPLANIN(TEI)	90%
TETRACYCLINE(TE)	90%
LINEZOLID(LZ)	88%
NITROFURANTOIN(NIT)	88%
HLS	58%
ERYTHROMYCIN(E)	46%
NORFLOXACIN(NX)	46%
HLG	40%
DOXYCYCLINE(DO)	38%
CIPROFLOXACIN(CIP)	26%
PENICILLIN(P)	12%
AMPICILLIN(AMP)	8%

This table shows the sensitivity pattern of Enterococcus. Vancomycin (VA) shows the highest sensitivity (94%), followed by Teicoplanin (TEI) 90%, followed by Tetracycline (TE) 90%, followed by Linezolid (LZ) 88%, followed by Nitrofurantoin (NIT) 88%, followed by HLS 58%, followed by Erythromycin (E) 46%, followed by Norfloxacin (NX) 46%, followed by HLG 40%, followed by Doxycycline (DO) 38%, followed by Ciprofloxacin (CIP) 26%, followed by Penicillin (P) 12%, followed by Ampicillin (AMP) 8%. The prevalence of Vancomycin Sensitive Isolates was 94%, and Vancomycin-Resistant Enterococcus was 6%.

DISCUSSION

The overall prevalence of Enterococcus was 25% which is similar to the results reported by Kamalasekaran et al. (2016) and Khanal et al. (2018). The highest prevalence of Enterococcus was in urine (80%), followed by blood 10%, followed by pus 6%, followed by vaginal swab 4%, which is similar to the results reported by Khanal et al. (2018).

Out of 50 Enterococcal isolates, 30(60%) were isolated from inpatients, and 20(40%) were isolated from the outpatients' department (OPD), which is

similar to the results reported by Khanal et al. (2018).

The distribution of positive Enterococcal isolates based on gender showed that more than half of the patients were females 35(70%), and only 15(30%) were males, which is similar to the results reported by Toru et al. (2018).

Distribution of positive Enterococcal isolates based on age group showed that the highest no. of patients were in the age-group of 31-40, 16 patients out of 50.

Distribution based on antibiotic resistance pattern of Enterococcus reported that Ampicillin showed the highest resistance 92%, similar to the results reported by Kamalasekaran et al. (2016), Penicillin 88% similar to the results reported by Khanal et al. (2018), Ciprofloxacin 74% identical to the results reported by Kamalasekaran et al. (2016).

Antimicrobial susceptibility testing of Enterococcus showed that the highest sensitivity was in Vancomycin (94%), Teicoplanin and Tetracycline (90%), Linezolid(88%). In the study, VRE was found to be 6% similar to the results reported by Khanal et al.

CONCLUSION

This study demonstrates that Enterococcus was considered low virulence earlier, but now they are becoming an important nosocomial pathogen. Due to the increased prevalence of multi drug resistant Enterococci with few isolates being resistant to almost all antibiotics tested, Vancomycin is the last drug of choice left to treat Enterococcal infections, but now days increased prevalence of VRE posing a serious therapeutic challenge. This condition warrants the implementation of an efficient infection control program and regular surveillance of Enterococci's antimicrobial resistance to establish a rational antibiotic policy for better management of Enterococcal infections.

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Conflicts of Interest: Nil Source of Funding: Nil

Citation: Singh S, Thokar MA, Srivastava S, Khan S, Dwivedi P, Rajbhar P. A Retrospective Antibiogram Study of Clinical Isolates of Enterococcus among Patients Attending at the IIMSR Hospital, Lucknow. National Journal of Medical and Allied Sciences 2021; 10 (2): 1-5

Date of Submission: 10-05-2020
Date of Acceptance: 20-05-2021