African Journal of Pharmaceutical Research & Development

Vol. 7 No.1 pp.24-31 (2015)

PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *ENTEROCOCCUS SP.* ISOLATED FROM A NIGERIAN HOSPITAL

Ayeni FA[1, 3]*, Bello AE[1], Olley M[2]

1-Department of Pharmaceutical Microbiology, Collage of Pharmacy, Igbinedion University Okada
2-Medical Microbiology Unit, Igbinedion University Teaching Hospital Okada
3-Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Ibadan

ABSTRACT

Enterococci are bacteria that are increasingly noted for their occurrence in infections in various sites in the body. There is currently a treatment challenge for enterococci infections due to antibiotic resistant problem observed in the bacteria thereby warranting surveillance study in different locales of the world. The purpose of this study therefore is to study prevalence of enterococci in infections in a Nigerian hospital over a 2 months period and determine its resistance pattern. Eighty-six samples comprising of 12 Endocervical Swab (ECS), 18 High Vaginal Swab (HVS), 43 Urine Samples, 2 Wound Swabs, 7 Sputum and 4 Ear Swabs were obtained from samples of patients who were referred to Medical Microbiology Unit of Igbinedion University Hospital between April and May, 2010. The isolates were identified by biochemical methods, antibiotic susceptibility of isolates to ciprofloxacin, doxycycline, ampicillin, nitrofurantoin and chloramphenicol were determined by disk diffusion test according to NCCLS and MIC tests for ciprofloxacin, ampicillin and chloramphenicol were carried out for selected resistant strains by macro-dilution method. Six *Enterococcus* sp. were identified only from urine, endocervical and vaginal samples with the highest occurrence observed in urine samples (9.3%). The isolated strains were highly resistant to doxycycline (66.7 %) but susceptible to nitrofurantoin. The MIC result reveals that selected strains were also resistant to ciprofloxacin and chloraphenicol. The result from this study indicates that there is low occurrence of enterococci as causative agent of infections in the studied environment. This is in contrast to reports from other part of the world. It also indicates that the best antibiotic for treatment of enterococci infection is nitrofurantoin while doxycycline is not effective for treatment of enterococci infections in the studied environment.

KEYWORDS- Enterococci, Antibiotics, Resistance, Infections

INTRODUCTION

Enterococci are members of the intestinal flora of humans and animals and may also colonize the upper respiratory tract, biliary tracts and vaginas of otherwise healthy persons [1]. Clinically, the enterococci are commonly the infecting agents in urinary tract infections, biliary tract infections, bacteremia, cholecystitis, cholangitis, peritonitis, septicaemia, endocarditis, meningitis, wound infections and subacute bacterial endocarditis (SBE). Enterococci have been recognized as leading causes of nosocomial bacteremia, surgical wound infection and urinary tract infection. [2,3]. Enterococci now rank among the top three nosocomial bacterial pathogens [4].

Management of enterococcal infections is generally by the use of antibiotics. The organisms are ordinarily resistant to aminoglycosides, sulphonamides, cephalosporins monobactams, many penicillins (e.g. isoxazolyl- penicillins, ticarcillin and carbenicillin), and the older quinolones. [3]. Ayeni *et al*, [5] also reported the resistance of *Enterococcus faecium* strains to tobramycin, cefaclor, cefuxime, ceftazidime, miokamycin, fosfomycin and co-trimoxazole. Despite the many intrinsic resistances of enterococci, most enterococcal infections, until recently, could be treated with penicillin, ampicillin,

*Author for correspondence: *E-mail:* fa.ayeni@ui.edu.ng

vancomycin with or without an aminoglycoside and the newest fluoroquinolones.

Unfortunately, enterococci have now acquired resistance to these antibiotics and many other agents as a result of mutations (e.g., causing high-level resistance to streptomycin or to fluoroquinolones) or the acquisition of new gene(s). The combination of high-level resistance to ampicillin, vancomycin, and aminoglycosides is now fairly common among hospital-acquired *Enterococcus faecium* and has a major impact on therapeutic options. The resistance pattern also varies in different environment as a result of the use of antibiotics.

Prevalence of E*nterococcus spp* as a causative agent of various infections in developed countries is well documented [2, 6]. However, there are relatively few reports available from Nigeria. Therefore the objectives of this study are to assess the prevalence of *Enterococcus sp* as a causative agent of various infections in a hospital environment, to assess the resistance pattern of the isolated *Enterococcus sp* to different antibiotics and to determine the Minimum Inhibitory Concentration (MIC) of various antibiotics to *Enterococcus sp.*

MATERIALS AND METHODS

Collection and analysis of samples

Eighty-six samples comprising of 12 Endocervical Swab (ECS), 18 High Vaginal Swab (HVS), 43 Urine Samples, 2 Wound Swabs, 7 Sputum and 4 Ear Swabs were collected from patients at Igbinedion University Teaching Hospital, Okada, Nigeria by the staff of Medical Microbiology unit of the hospital between April and May, 2010.

The samples were microbiologically examined for the presence of *Enterococcus sp*. by inoculating on triplicate media plates i.e. McConkey agar (Lab M, UK), Blood agar (Lab M, UK) and Chocolate agar (Lab M, UK). Plates were incubated at 37^{0}C for 24 hrs in 5% CO_2..

Identification of Enterococcus Strains

Gram staining and catalase tests were performed on the isolates for preliminary identification. A drop of 3% H_2O_2 was added to a slide, by means of a loop wire, an innoculum of 18 hr old culture of each organism was made on the drop. The mixture was observed for the presence or absence of bubbles.

The following tests were done to confirm the identities of the isolates. Colonial morphology on solid media were studied by streaking an 18 hr old culture of the micro-organism on McConkey agar plate, Chocolate and Muller Hinton agar (Lab M, UK) plates and incubated for 48 hr at 37^{o}C. Isolates were further grown on Urea agar medium which was prepared by adding .5 ml of 40% urea to 95 ml of sterilized Urea agar base. Growth in the presence of salt was detected by streaking the isolates on sterilized McConkey without salt but with 6.5% salt (NaCl) added to the broth

Antibiotic susceptibility tests

The susceptibility of the bacteria to different antibiotics was tested according to a breakpoint method which agrees with standard indicated by National Committee for Clinical Laboratory Standard [7], using standard antibiotic discs (Oxoid, UK). One millimeter of 24 hr old bacterial culture of approximately 10^8 cfu/ml which is equivalent to McFarland standard 0.5 was inoculated into solidified Mueller Hinton agar plates by spread plate method. Five different standard antibiotic disks namely: ciprofloxacin 5 µg, doxycycline hydrochloride 30 µg, ampicillin 10 µg, nitrofurantoin 300 µg and chloramphenicol 30 µg were placed at least 2 cm apart on the agar plates using a sterile forceps. The plates were then incubated for 24 hr at 37^{o}C and examined for clear zones of inhibition around the discs. The diameter of inhibition were measured and compared with standard zones to determine resistance as instructed by the manufacturer of the antibiotic discs (Oxoid, UK).

Determination of Minimum Inhibitory Concentration (MIC)

Stock solution of Ciprofloxacin (Bal Pharma, China), Ampicillin (Shijiazhuang Pharma, China) and Chloramphenicol (Maxheal Pharmaceutical, India) were prepared to concentration of 50 µg/ml, 100 µg/ml and 300 µg/ml respectively.

The MIC of the antibiotics to the bacterial strains were determined by adding 1 ml of stock antibiotic solution to 9 ml of McConkey broth thereby reducing the concentration by 1/10. Five (5) ml of the medium/antibiotics solution was added to another 5 ml of McConkey broth thereby halving the antibiotic concentration. This was repeated until the least desired antibiotic concentration was obtained. One millimeter of 24 hr old bacterial culture of approximately 10^8 cfu/ml which is equivalent to McFarland standard 0.5 was inoculated into each antibiotics/medium mixture and incubated at 37^{o}C for 24 hr. The tubes were examined for growth which is observed by colour change in the McConkey broth (from purple to yellow) and turbidity. The least concentration at which no

bacterial growth can be discerned is selected as the MIC.

RESULTS

Six *Enterococcus* sp. were identified from the 86 samples. The *Enterococcus* strains were all Gram positive, catalase negative, grows in 6.5% salt with colour change from purple to black in McConkey broth, grows in the presence of urea with colour change from yellow to pink on urea agar medium and caused no haemolysis on blood agar. These characteristics confirm the bacteria as *Enterococcus* sp. The occurrence of *Enterococcus* sp. as causative agents of infections in the studied hospital is low. No enterococci were isolated from wound, sputum, and ear thereby indicating they were not the causative agent of the infections in these sites (Table I). They were only isolated from urine with 9.3% frequency of occurrence, endocervical with 8.3% frequency of occurrence and vagina with 5.6% frequency of occurrence (Table I).

Antibiotic susceptibility tests were done on the six *Enterococcus* sp by disc diffusion method. Most strains were very sensitive to nitrofurantoin (16.7%) and to a lesser extent chloramphenicol (33.3%), while there was high resistance to doxycycline (66.7%) and moderate resistance were observed to ciprofloxacin and ampicillin (50%) (Table II, Table III). Each strain had varied overall resistance pattern that ranges between 20% and 80%. *Enterococcus* sp. AMZ 020 was very sensitive to most tested antibiotics while *Enterococcus* sp. AMZ 066 was very resistant to tested antibiotics (Table II, Table III)

The Minimum Inhibitory Concentrations of the various antibiotics to some selected resistant *Enterococcus* strains were determined by broth macrodilution. The MIC result confirms the observed resistance in disc method. The selected strains were highly resistant to ciprofloxacin and chloraphenicol. *Enterococcus* sp. AMZ 021, 059 and 020 were very resistant to the concentrations of Ciprofloxacin used, though *Ent.* sp. AMZ 020 had MIC less than 5 µg/ml. When tested against chloramphenicol, *Ent.* sp. AMZ 066 showed resistance with MIC ≥30 µg/ml while *Ent.* sp. AMZ 018 had MIC of 15 µg/ml (Table IV).

DISCUSSION

The importance of this study lies in describing the prevalence of enterococi as causative agent of infections in a Nigerian hospital and also their sensitivity to various antibiotics in order to determine the best antibiotics for treatment of enterococcal infections.

The prevalence of the enterococcal isolates is relatively low in this study, which could be an indication that *Enterococcus sp*. are not often responsible for infections in this part of Nigeria. This correlates with a study which says that enterococci are responsible for 2-4% of urinary tract infections in general practice and 3-5% in hospital [8]. However enterococci have been identified in a report from the National Infections Surveillance in U.S. hospitals as the second most frequent nosocomial pathogen that plays a crucial role in 12% of all hospital acquired infection [9]. Also, Akhi *et al*, [10] reported that enterococci are mainly responsible for nosocomial infections in Iran. The few enterococci isolated from this study are from urogenital tract therefore, the enterococci used in this study were mainly responsible for urogenital infections and no other infections in the environment.

Table 1. Percentage Occurrence of *Enterococcus* sp. from Different Samples

Samples	No of Samples	Isolated *Enterococcus* sp. in samples	% occurrence
Endocervical Swab	12	1	8.3%
High Vaginal Swab	18	1	5.6%
Urine	43	4	9.3%
Wound Swab	2	0	0%
Ear Swab	4	0	0%
Sputum	7	0	0%

Table II. Antibiotic Susceptibility Pattern of Different *Enterococcus* Strains

Enterococcus Strains	Tested Antibiotics				
	Ampicillin (B.P. ≤ 11)	Ciprofloxacin. (B.P. ≤ 15)	Chloramphenicol (B.P. ≤ 12)	Nitrofurantoin (B.P.≤14)	Doxycycline. (B.P.≤ 12)
Enterococcus sp AMZ 001	10 mm R	25 mm S	13 mm S	16 mm S	0 mm R
Enterococcus sp. AMZ 018	25 mm S	27 mm S	9 mm R	19 mm S	0 mm R
Enterococcus sp. AMZ 021	0 mm R	15 mm R	13 mm S	20 mm S	0 mm R
Enterococcus sp. AMZ 020	30 mm S	0 mm R	22 mm S	33 mm S	25 mm S
Enterococcus sp. AMZ 066	10 mm R	30 mm S	11 mm R	12 mm R	11 mm R
Enterococcus sp. AMZ 059	12 mm S	0 mm R	21 mm S	30 mm S	26 mm S

Enterococcus Strains	Tested Antibiotics				
	Ampicillin (B.P. ≤ 11)	Ciprofloxacin. (B.P. ≤ 15)	Chloramphenicol (B.P. ≤ 12)	Nitrofurantoin (B.P.≤14)	Doxycycline. (B.P.≤ 12)
Enterococcus sp AMZ 001	10 mm R	25 mm S	13 mm S	16 mm S	0 mm R
Enterococcus sp. AMZ 018	25 mm S	27 mm S	9 mm R	19 mm S	0 mm R
Enterococcus sp. AMZ 021	0 mm R	15 mm R	13 mm S	20 mm S	0 mm R
Enterococcus sp. AMZ 020	30 mm S	0 mm R	22 mm S	33 mm S	25 mm S
Enterococcus sp. AMZ 066	10 mm R	30 mm S	11 mm R	12 mm R	11 mm R
Enterococcus sp. AMZ 059	12 mm S	0 mm R	21 mm S	30 mm S	26 mm S

NOTE: S = Susceptible to the antibiotics. R = Resistant to the antibiotics. B.P. = Standard Break Point to determine resistance as instructed by the manufacturer of the disc (Oxoid, UK).

Table III. Percentage Antibiotic Resistance Pattern of different *Enterococcus* strains.

Organism	% Resistance (A)	Antibiotics	% Resistance (B)
Enterococcus sp AMZ 001	40%	Ampicillin	50%
Enterococcus sp. AMZ 018	40%	Ciprofloxacin	50%
Enterococcus sp. AMZ 021	60%	Chloramphenicol	33.3%
Enterococcus sp. AMZ 020	20%	Nitrofurantoin	16.7%
Enterococcus sp. AMZ 066	80%	Doxycycline.	66.7%
Enterococcus sp. AMZ 059	40%		

B; -% resistance to of all Enterococcus strains to each antibiotic
A; -% resistance of each Enterococcus strain to all tested antibiotics.

Table IV. Minimum Inhibitory Concentration of Three Antibiotics to Selected Resistant *Enterococcus* Strains

Antibiotics	*Enterococcus* strains	MIC ((µg /ml)
Ciprofloxacin	*Enterococcus* sp AMZ 021	>5 µg/ml
	Enterococcus sp AMZ 059	>5 µg/ml
	Enterococcus sp AMZ 020	5µg/ml
Chloramphenicol	*Enterococcus* sp AMZ 066	>30 µg/ml
	Enterococcus sp AMZ 018	15 µg/ml
Ampicillin	*Enterococcus* sp AMZ 001	2.5 µg/ml
	Enterococcus sp AMZ 021	<1.25µg/ml
	Enterococcus sp AMZ 066	2.5 µg/ml

Note: - Breakpoint for Ampicillin is 8µg/ml
Breakpoint for Chloramphenicol is 8µg/ml
Breakpoint for Ciprofloxacin is 4µg/ml

The result from this study showed that enterococci are susceptible to nitrofurantoin and chloramphenicol. A similar susceptibility was found in a multi-centre study at United States and Canada, which not only revealed that the enterococci are susceptible to linezolid, nitrofurantoin and chloramphenicol but also these antibiotics have been recommended for urinary tract infection caused particularly by vancomycin resistant enterococci. [11]. Abdulla and Abdulla, [12] also reported high sensitivity of enterococci to nitrofurantoin and chloramphenicol while highly resistant to ampicillin, ciprofloxacin and doxycycline as reported in this study.

Enterococci have been noted for their resistance to a variety of antimicrobials due to intrinsic or acquired resistance [3]. Resistance to antimicrobials in enterococci has been found throughout the world and is generally recognized as associated with antimicrobial use [13]. It is generally accepted that the main risk factor for the increase in the antibiotic resistance is an extensive use of antibiotics. This has lead to the emergence and dissemination of resistant bacteria and resistance genes in animals and humans. In both populations antibiotics are used for therapy and prophylaxis of infectious diseases [10].

The resistant rate to ampicillin (57.66%) in enterococcal isolates in a study done in Iran was close to the resistance rate to ampicillin in Ireland (51%). [14] However resistance rate to ampicillin reported by Mathur *et a*l., [15] in India (66%) was higher than both results which is close to the result gotten from this study (50%). Resistance to ampicillin is attributed to mutations in the gene coding for PBP-5, decreasing the affinity for penicillins in this protein [3].

The high resistance rate of doxycycline observed in this study is alarming. Tetracyclines are over-the-counter (OTC) drugs which have been seriously abused because of their broad-spectrum of activities and affordability in terms of cost. The extensive uses of tetracyclines have often led to an emergence of resistant bacteria [13, 16]. Kayser, [3] reported that enterococcal resistance to tetracyclines may be acquired through exchange of resistance encoding genes by conjugation mediated either by conjugative transposons, pheromone-responsive conjugative plasmids or broad host range conjugative plasmids.

Resistance to fluoroquinolones has increased markedly since their introduction for UTI treatment. Many studies worldwide reported a clear increase in ciprofloxacin resistance. In China, from 1998 to 2002 the incidence of ciprofloxacin resistance increased steadily from 46.6% to 59.4% [17]. In Spain, it was 14.7% [18] and in Bangladesh, it was 26.0 % [19] which is lower than the value seen in this study (50%).

The MIC result generally agrees with the disc diffusion result except in *Enterococcus* sp. 021 resistance to ampicillin. The high MIC observed in *Enterococcus* sp AMZ 021, *Enterococcus* sp AMZ 059 (to ciprofloxacin) and *Enterococcus* sp AMZ 066 (to chloramphenicol) warrant further investigation of the presence of ciprofloxacin and chloramphenicol resistant genes in the affected isolates.

CONCLUSION

Current antibiotic therapies for treatment of enterococcal infection should be based on local pattern of resistance. From this study, nitrofurantoin could be the drug of choice for the treatment of enterococcal infections. The enterococcal resistance to doxycycline doesn't come as a surprise because in recent years, these Over the Counter drugs have been severely abused, hence, their resistance. It is recommended that there should be strict rules governing the use of antibiotics in Nigeria such that no antibiotics should be sold without a prescription and also doctors should not be quick to prescribe antibiotics for mild infections that could be treated without them. These would help a great deal in reducing the rapid emergence of growing resistance of enterococci to various antibiotics they were sensitive to before such as ciprofloxacin and ampicillin. These resistant enterococci are formidable nosocomial pathogen thereby posing therapeutic dilemmas for clinicians. Thus it is crucial for laboratories to provide accurate antimicrobial resistance patterns for enterococci so that effective therapy and infections control measures can be initiated.

REFERENCES

1. Gold, HS. Vancomycin- resistant enterococci: mechanisms and clinical observations. *Clin Infec Dis* 33, 2001, 210-219

2. Murray BE. Vancomycin resistant enterococcal infections. *New Engl J Med* 342, 2000, 710-721.

3. Kayser FH. Safety aspects of enterococci from the medical point of view. *Int J Food Microb*. 88, 2003, 255-262

4. Domann E, Hain T, Ghai R, Billion A, Kuenne C, Zimmermann K, *et al*., Comparative genomic analysis for the presence of potential enterococcal virulence factors in the probiotic *Enterococcus faecalis* strain Symbioflor 1. Int J Med Microbiol. 297, 2007, 533-539
5. Ayeni FA, Adeniyi BA, Ogunbanwo ST, Tabasco R, Paarup T, Peláez C and Requena T. Inhibition of uropathogens by lactic acid bacteria isolated from dairy foods and cow's intestine in western Nigeria. *Arch Microbiol*. 191 (8), 2009, 639-648
6. Wong AHM, Wenzel RP and Edmond MB. Epidermiology of bacteriuria caused by vancomycin-resistant enterococci: a retrospective study. *Am J Infec Cont* 28, 2000, 277-281
7. NCCLS. Performance Standards for antimicrobial susceptibility testing; Twelfth Informational Suppliment. NCCLS document M100-S12 ISBN Pennsylvania, 2002 1-56238-454-6].
8. Gruneberrg, N. Antibiotic sensitivities of urinary pathogens, 1971-1978. J Clin Path. 33, 1980, 853.
9. Schaberg DR, Culver DH, Gaynes RP. Major trends in the microbial etiology of nosocomial infection. *Am J Medicine*. 91, 1991, 121-125.
10. Akhi MT, Farzaneh F, Oskowei M. Study of enterococcal susceptibility patterns isolated from clinical specimens in Tabriz, Iran. *Pak J Med Sci.* 25 (2), 2009, 211-216.
11. Zhanel GG, Laing NM, Nichol KA. *et al*. Antibiotic activity against urinary tract infection (UTI) isolates of vancomycin-resistant enterococci (VRE): results from the 2002 North American Vancomycin Resistant Enterococci Susceptibility Study (NAVRESS). *J Antimicrob Chemother* 52, 2003, 382-388.
12. Abdulla FE and Abdulla EM. Antibiotic options for *Enterococcus faecalis* infections. *Pak J Med Sci.* 22 (3), 2006, 286-290.
13. Obeng AS, Rickard H, Ndi, O, Sexton M, Barton M. Comparison of antimicrobial resistance patterns in enterococci from intensive and free range chickens in Australia. *Avian Pathology* 42 (1), 2013,45-54
14. Lavery A, Rossney AS, Morrison D, Power A, Keane CT. Incidence and detection of multi-drug resistant enterococci in Dublin hospitals. *J Med Microbiol* 46, 1997, 150-156.
15. Mathur P, Kapil A, Chandra R, Sharma P, Das B. Antimicrobial resistance in *Enterococcus faecalis* at a tertiary care centre of northern India. *Indian J Med Res* 118, 2003, 25-28.
16. Chopra I. and Roberts M. Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial esistance. *Microbiol. and Mol. Bio. Rev*., 65, 2001, 232-260.
17. Shao HF, Wang WP, Zhang XW, Li ZD. Distribution and resistance trends of pathogens from urinary tract infections and impact on management. Zhonghua Nan Ke Xue. 9(9), 2003, 690–692.
18. Kahlmeter G. An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO.SENS Project. *J Antimicrob Chemother* 51(1), 2003, 69–76.
19.Iqbal J, Rahman M, Kabir MS, Rahman M. Increasing ciprofloxacin resistance among prevalent urinary tract bacterial isolates in Bangladesh. *Jpn J Med Sci Biol.* 50(6), 1997, 241–250.