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Clinical Science

Multiple Antibiotic Resistance Patterns of Enterococcus Species Isolated From Ahmadu Bello University Teaching Hospital, Zaria

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ABSTRACT [ENGLISH/ANGLAIS]

This study demonstrated the multiple antibiotic resistant patterns of *Enterococcus* species isolated from patients attending Ahmadu Bello University Teaching Hospital, Zaria. A total of 310 samples comprising 110 [35.5%] stool, 100 (32.3%) urine and 100 (32.3%) wound swabs were analysed. The clinical samples yielded a total of 143 (46.1%) enterococcal isolates. All the isolates (100%) were susceptible to vancomycin. Thirty two (22.4%) strains were resistant only to single antibiotics. Seventy eight (54.6%) showed multiple resistance to ampicillin, chloramphenicol, gentamycin, erythromycin and tetracycline. However, 33 (23%) of the isolates were susceptible to all the antibiotics tested. The results indicated that a significant percentage of isolates were resistant to different antibiotics used suggesting the need for control strategies to avoid dissemination of resistant isolates and emphasizing the importance of surveillance for the detection of emerging resistance traits.

Keywords: Enterococcus species, vancomycin, resistance, susceptible

RÉSUMÉ [FRANÇAIS/FRENCH]

Cette étude a démontré les multiples schémas antibiotiques résistants d'espèces *Enterococcus* isolées de patients qui fréquentent l'Université Ahmadu Bello Teaching Hospital, Zaria. Un total de 310 échantillons comprenant 110 [35,5%] selles, 100 (32,3%) d'urine et 100 (32,3%) prélèvements plaies ont été analysés. Les échantillons cliniques a produit un total de 143 (46,1%) isolats d'entérocoques. Tous les isolats (100%) étaient sensibles à la vancomycine. Trente-deux (22,4%) souches étaient résistantes aux antibiotiques seuls unique. Soixante-dix huit (54,6%) ont montré une résistance multiple à l'ampicilline, le chloramphénicol, gentamicine, l'érythromycine et la tétracycline. Toutefois, 33 (23%) des isolats étaient sensibles à tous les antibiotiques testés. Les résultats indiquent qu'un pourcentage significatif des isolats étaient résistants à des antibiotiques différents utilisés suggérant la nécessité de stratégies de contrôle pour éviter la diffusion de souches résistantes et soulignant l'importance de la surveillance pour la détection de caractères de résistance émergents.

Mots-clés: Enterococcus, vancomycine, résistance, susceptible

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INTRODUCTION

Enterococci are facultative anaerobic Gram-positive cocci that appear in pairs or short chains. They share their morphology and Lancefield antigenicity with group D streptococci. The genus Enterococcus includes at least 17 species, distinguished on the basis of pigment production, motility, and ability to produce acids from various carbohydrates with Enterococcus faecalis (90-95%) and E. faecium (5-10%) the common commensals in the intestine of man [1]. They are tolerant of wide environmental conditions such as extreme temperature (10-45 °C), pH (4.5-10.0), and high salt concentration [2].

Recent years have witnessed increased interest in enterococci not only because of their ability to cause serious infections such as endocarditis, bacteremia, intra-abdominal and urinary tract infection (UTI), but also because of their increasing resistance to many antimicrobial agents [3]. Enterococcus species are not considered primary pathogens, but because of their ability to acquire high-level resistance to antimicrobial agents, they have emerged as nosocomial pathogens worldwide [4]. Maraha et al. [5] noted that *Enterococci* are frequent causative agents of both nosocomial and community acquired infection in transplant patients, including

surgical site infections. Increase use of antibiotics such as aminoglycoside, cephalosporin, quinolones and glycopeptides contribute to resistance in enterococci [6].

The aim of this work was to determine the number of antibiotics to which each of the isolated organisms was resistant to.

MATERIALS AND METHODS

The study was carried out at Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, a tertiary health facility that serves as a referral centre for specialized health services for Kaduna State, and some states in Northern part of the country. Ethical approval was granted by the ethical board of the hospital for this study.

A total of 310 samples of stool, urine and wound swabs were collected from the department of Medical Microbiology of the, ABUTH Shika-Zaria. All the specimens were aseptically collected into sterile containers and immediately transported to the laboratory for analysis. The isolates were identified by cultural characteristics, Gram's stain, motility testing and biochemical test such as catalase negativity, growth on and blackening of bile aesculin azide agar, growth at 45 ^{0}C, growth in 40% bile agar, growth in 6.5% sodium chloride broth, H_2S production, fermentation of various sugars such as Arabinose, Fructose, Glucose, Lactose, Mannitol, Sorbitol, Sorbose, and Sucrose.

Antimicrobial Susceptibility Testing: Susceptibility to all antimicrobial agents was determined by the standard disc diffusion method according to CLSI [7] guideline on Mueller Hinton agar. The following antibiotics discs (oxoid) were used: Vancomycin (30ug), Erythromycin (15ug), Tetracycline (30ug), Gentamycin (10ug), Ampicillin (10ug) and Chloramphenicol (30ug).

RESULTS AND DISCUSSION

Of the 310 samples analysed, 143 yielded enterococcal isolates giving 46.1% prevalence as shown in table 1. Stool yielded the highest with 109 (76.2%) isolates; Urine yielded 27 (18.9%) isolates while wound swabs yielded 7 (4.9%) isolates.

The strains isolated showed 5 distinct patterns of resistance to the antibiotics used as shown in table 2. For resistance to single antibiotics, seventeen strains were resistant to Tetracycline, 3 to Gentamicin, 11 to Erythromycin and 1 to Ampicillin indicating that a total of 32 (22.4%) strains were resistant. The number of strains that showed resistance to 2 different kinds of antibiotics tested were 38 (26.6%); those that showed resistance to three antibiotics tested were 22 (15.4%); while those that showed resistance to 4 different antibiotics tested were 14 (9.8%). Four (2.8%) of the strains were resistant to 5 different kinds of antibiotics tested. Of the 143 isolates, only 33 (23%) were susceptible to all the antibiotics tested. General resistance was observed to all the antibiotics tested except to vancomycin giving a percentage of 54.6. However, the inability to isolate VRE could be attributed to non-availability or scarcity of vancomycin in most pharmacies and chemist stores in Kaduna State, Nigeria which makes patients not accessible to it and therefore not subjected to abuse/misuse as noted by Oduyebo et al. [8]. Nonetheless, there is a need for constant monitoring as sporadic isolates have recently been reported as vancomycin resistant [9]. The relative high susceptibility to ampicillin in this work is of great importance since it is the drug of choice in the treatment of enterococcal infections. This enterococcal susceptibility agreed with the work of Jabalameli et al. [10] and Bonadio et al. [11]. However resistance to gentamicin, an aminoglycosides, in this study, is of great concern since it eliminates synergy with cell wall active antibiotics such as ampicillin use in combination for the treatment of enterococcal infections such as endocarditis [12].

The isolates that were resistant to tetracycline, erythromycin and gentamicin were more in occurrence than to other antibiotics as a result of frequent use of these antibiotics in this part of the world.

The multiple resistance level (54.6%) in this work is high and as such should be checked as *Enterococcus* species easily acquire and transfer resistance gene from neighbouring microorganisms or environment [4]. Fourteen (9.8%) strains exhibited resistance to four different antibiotics tested while 4 (2.8%) were resistant to five different antibiotics tested. If these strains disseminate into the community, the level of multi-resistance genes spread to members of the same species and to other bacteria would portend danger. Multidrug resistance as a result of misuse of antibiotics cause serious problems in the treatment of patients suffering from nosocomial infection due Enterococcus species. Some of the risk factors for multiple drug resistance enterococci include haemodialysis, receipt of corticosteroids, anti-neoplastic agents or total parenteral nutrition, surgery, severity of illness, antimicrobial administration, indwelling bladder catheters, neutropenia and mucositis which lead to prolong hospital stay [13, 14].

Resistance of enterococci to many antibiotics could be intrinsic or acquired. Unlike acquired resistance, intrinsic resistance is based in chromosomal genes, which typically are nontransferable. Enterococci often acquire antibiotic resistance through exchange of resistance-encoding genes carried on conjugative transposons, pheromone-responsive plasmids, and other broad-host-range plasmids.

Resistance in bacteria have steadily developed as a result of the widespread use of antibiotics 50 years ago [15]. The past two decades have witnessed the rapid emergence of Multi-Drug Resistant (MDR) enterococci and the consequent increase report of nosocomial infections. Knowledge of the pattern of resistance is therefore necessary to formulate the treatment guideline for infections caused by enterococcus species. Therefore, to control emergence of resistance, there is a need for prudent use of antibiotics both in the hospital and clinics and constant monitoring of MDR enterococci.

Table 1: This table shows *Enterococcus* species isolated from clinical samples at ABUTH, Zaria

Specimen	No. Tested (%)	No. of Isolates (%)
Stool	110 (35.5)	109 (76.2)
Urine	100 (32.3)	27 (18.9)
Wound	100 (32.3)	7 (4.9)
Total	310	143 (46.1)

Table 2: This table shows Multiple Antibiotic Resistance Pattern of *Enterococcus* species isolated from clinical specimens at ABUTH Zaria

No. of antibiotics	No. of isolates with pattern	Total number (%)	Resistance patterns/phenotypes
1	17	32 (22.4%)	TET
	3		GEN
	11		ERY
	1		AMP
2	9	38 (26.6%)	TET GEN
	12		ERY TET
	3		GEN AMP
	4		TET CHL
	9		ERY GEN
	1		TET AMP
3	8	22 (15.4%)	ERY TET AMP
	7		ERY TET CHL
	2		TET GEN AMP
	4		ERY TET GEN
	1		TET GEN CHL
4	12	14 (9.8%)	ERY TET GEN CHL
	1		ERY TET GEN AMP
	1		ERY TET AMP CHL
5	4	4 (2.8%)	ERY TET GEN AMP CHL

TET – Tetracycline; GEN – Gentamicin; ERY – Erythromycin; AMP – Ampicillin; CHL – Chloramphenicol

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CONFLICT OF INTEREST

No conflict of interests was declared by authors.