

Fecal Carriage of Vancomycin-Resistant Enterococci among Hospitalized Patients in Khartoum Sudan

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ABSTRACT

Background: Vancomycin-resistant Enterococci have emerged as important nosocomial pathogens worldwide. These organisms are often resistant to multiple antimicrobial agents limiting the number of antimicrobial treatment options.

Aim: This study aimed to isolate and identify the vancomycin-resistant enterococci from fecal specimens of hospitalized patients in Soba hospitals- in Khartoum, Sudan.

Methods: The current is a descriptive cross-sectional laboratory-based study. 100 hospitalized patients were selected randomly. A fecal specimen was collected from each participant and inoculated on Kanamycin esculin azide agar and incubated at 37°C overnight. Isolated colonies were identified using conventional microbiological methods. Antimicrobial susceptibility testing was determined by using the Kirby-Bauer disc diffusion method. Vancomycin resistance was detected by using vancomycin screening agar. Collected data were analyzed using Statistical Package for Social Science, version 22, *P. value* <0.05 was considered statistically significant

Results: Out of 100 fecal specimens, 89 *Enterococcus spp.* were isolated. 1 out of 89 Enterococci (1.1%) were found vancomycin resistance. Resistant to Ceftriaxone was 100%, Rifampicin was 98.9% and streptomycin was 89.9%.

Conclusion: There was a low prevalence of a vancomycin-resistant and wide range of other antimicrobial agents' resistance with an increase in multi-drug resistant strains of enterococci.

Keywords: Antimicrobial; Enterococci; Multidrug Resistant; Vancomycin

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I. INTRODUCTION

Enterococci are opportunistic and nosocomial human pathogens that can cause urinary tract infections, burn wound and surgical site infections, bacteremia and sepsis, endocarditis, cholecystitis, peritonitis, neonatal

meningitis, and others ⁽¹⁾. *E. faecalis* is one of the most frequently isolated microbial flora in the human gastrointestinal tract, while *E. faecium* isolated with less frequency ⁽²⁾.

Enterococci are part of the microbial flora of the gastrointestinal tract of animals and humans and can

be released into the environment through fecal materials⁽³⁾. Residues of Antimicrobials and Antimicrobials resistance genes were detected from human environments and farms and they must be considered as important pollutants⁽⁴⁾. Dissemination of resistant genes among microbial flora and pathogenic bacteria in sewage can play a major role in the development of MDR isolates⁽⁵⁾. Methods to reduce the amount of antimicrobial agents and a load of resistant bacteria in wastewater originating from hospitals and farms include the optimization of disinfection procedures and management of wastewater and manure⁽⁶⁾. These resistant isolates may be transmitted to humans through contaminated water, food, or vegetables⁽¹⁾.

The emergence of multidrug-resistant (MDR) Enterococci has become a serious problem in healthcare settings worldwide⁽⁷⁾. The intrinsic resistance to various antimicrobials and dissemination of resistant genes by horizontal transfer is currently thought to play a major role in the development of MDR Enterococci⁽⁸⁾. These MDR isolates are associated with increased mortality and costs due to prolonged hospitalization, need for surgery, and prolonged treatment with antimicrobials⁽⁹⁾. A common regimen for the treatment of serious Enterococcal infections is the synergistic combination of cell wall inhibitors such as penicillin, Ampicillin, or vancomycin with Aminoglycosides such as streptomycin or Gentamicin⁽¹⁰⁾.

However, Vancomycin Resistant Enterococci (VRE) has caused significant problems for Antimicrobial therapy⁽¹⁰⁾. VRE were first isolated in the UK and France in 1986. Since that time, hospital outbreaks of VRE have been reported in the UK, Ireland, Italy, and Finland⁽¹¹⁾. VRE carriage has also been reported among out-patients in Germany and patients that newly admitted to hospitals in France⁽¹²⁾. According to van der Auwera *et al.*, fecal cultures of 11 (28%) out of 40 healthy individuals who were not health care staff and who had not taken Antimicrobials in the previous year gain a heterogeneous collection of VRE⁽¹³⁾. In the

Netherlands, 5 to 10% of healthy people were colonized with VRE and a study in a cattle-rearing region in France revealed that 11.8% of non-hospitalized people and 37% of hospitalized patients were carriers of VRE⁽¹⁴⁾.

II. METHODS AND MATERIAL

This descriptive cross-sectional laboratory-based study was performed in Soba hospital- in Khartoum, Sudan from September to January 2022. 100 hospitalized patients were selected randomly. Patient's data including age and gender were collected from lab records. The fecal specimen was collected from each participant

Enterococci isolation and Identification: Fresh collected fecal specimens were inoculated on Kanamycin Esculin Azide agar (Merck, Germany) and incubated at 37°C for 24 h. Then, the black colonies were examined to certify the gram-positive cocci through the gram staining technique. Bacterial identification was done by colonial morphology, identification was based on gram stain litmus, milk, and bile esculin hydrolysis test. Standard strains for quality control were used, *E. faecalis* ATCC 2921, *E. faecalis* ATCC 51299⁽¹⁵⁾

Antimicrobial susceptibility testing: Assessment of determining susceptibility to antimicrobial was performed by Kirby-Bauer agar disk diffusion assay. The susceptibility of enterococci was determined for the following Antimicrobials; vancomycin (30 µg), streptomycin (30 µg), erythromycin (15 µg), Ampicillin (10 µg), ciprofloxacin (5 µg), and Chloramphenicol (30 µg), Rifampicin (2 mg), Gentamicin (10 mg), Cefepime (50 mg), To estimate the sensitivity of isolates to Antimicrobials, the size of inhibition zone was measured and compared against a reference standard chart of the Clinical and Laboratory Standards Institute (CLSI)⁽¹⁶⁾.

Vancomycin detection: After Direct colony suspension to obtain 0.5 McFarland turbidity was prepared. Using a micropipette, a 10-µL drop was

spotted on the agar surface. The plates were incubated at 35°C aerobically for 24 hours. > 1 colony = reported as Presumptive vancomycin resistance, QC strain ATCC 29212, *E. faecalis* ATCC 51299 were used (17).

III. RESULTS AND DISCUSSION

3.1 Enterococci carriage rate: In this study, a total of 100 fecal specimens were collected from hospitalized patients, 89 of them were carry fecal *Enterococcus spp.*

3.2. Age distribution: The age range of the study group was between 20 and 78 years and the median age was 41 years and above 40 years age.

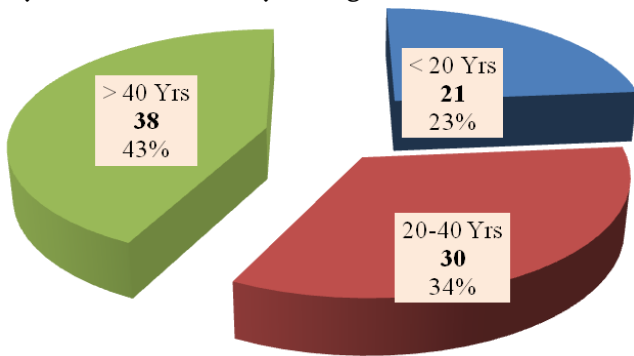


Figure 3.1. Age distribution among the study group No. 89

3.3. Gender distribution: About 62% of the study population were males and 38.2% were females.

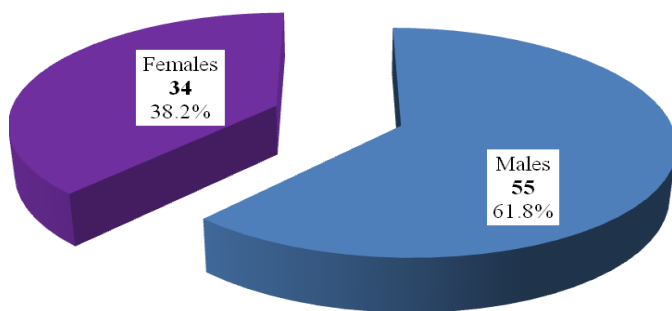


Figure 3.2. Gender distribution among the study group No. 89

3.4 Susceptibility to Antimicrobial Agent: The enterococci isolated were highly resistant 100% to Ceftriaxone and 98.9% to Streptomycin and

Rifampicin as in table 3.1.

Table 3.1. Antimicrobial resistance among *Enterococcus spp.* isolates No. 89

Antimicrobial	Resistance	
	No.	%
Gentamicin	76	85.4
Erythromycin	78	87.6
Ampicillin	66	74.2
Ciprofloxacin	60	67.4
Streptomycin	80	89.9
Rifampicin	88	98.9
Ceftriaxone	89	100.0
Chloramphenicol	03	03.4
Cefepime	89	100.0

As listed in table (4), 100% of the fecal enterococci showed multi-drug resistance to the tested antimicrobials (strain resists 4 or more antimicrobials)

Table (3.2): Multi-drug resistance among *Enterococcus spp.* isolates No. 89

Resistance	No. (%)
Fully sensitive	00 (00.0)
Resistant to 1 agent	00 (00.0)
Resistant to 2 agents	00 (00.0)
Resistant to 3 agents	00 (00.0)
Resistant to 4 agents	03 (03.3)
Resistant to 5 agents	04 (04.5)
Resistant to 6 agents	14 (15.8)
Resistant to 7 agents	31 (34.9)
Resistant to 8 agents	37 (41.5)
Resistant to 9 agents	00 (00.0)
Total	89 (100)

3.5 Vancomycin screening: Vancomycin screen agar of enterococci isolates showed that 1 (1.1%) strain was found vancomycin-resistant. This VRE was multi-drug resistant and resistant to Rifampicin, Chloramphenicol, and Streptomycin.

Discussion

Enterococci have emerged in the last 3 decades, as well as acquired resistance to many antimicrobials such as Vancomycin, resulting in multi-drug-resistant strains, which become increasingly in the hospital setting ⁽¹⁸⁾. Vancomycin has been the most reliable and available therapeutic agent against serious life-threatening infections caused by MDR Enterococci and Staphylococci as the last line drug, but unfortunately, decreases in Vancomycin susceptibility of these isolates were recently reported in many countries.

In this study out of 100 fecal specimens, Enterococci strains were isolated from 89%, this high Enterococcal fecal carriage rate agrees with Mogadam and Yassin in Khartoum-Sudan (2019) (87%) ⁽¹⁹⁾, Hijazi and his colleagues in Gaza-Palestine (2009) (94%) ⁽²⁰⁾

CLSI recommends the use of 6 µg/ml vancomycin-containing agar to detect vancomycin resistance among Enterococci isolates and only one (1.1%) was VRE. This low resistant rate agrees with a study done in Kuwait that reported that 1.9% of enterococci were VRE ⁽²¹⁾. Low vancomycin was also reported in Jordan (4%) ⁽²²⁾ and Iran (4%) ⁽²³⁾. On the other hand, a high rate of VRE was reported in another study from Iran (16%) ⁽²⁴⁾, VRE are reported worldwide the epidemiology of this strain varies widely in different geographical areas and populations.

Our isolated strains showed high resistant rates to a wide range of antimicrobials, a very high resistance rate (85-100%) was reported against Gentamicin, Erythromycin, Streptomycin, Rifampicin, Ceftriaxone, and Cefepime. A high resistance rate (67 and 74 respectively) was reported against Ciprofloxacin and Ampicillin; while Chloramphenicol showed activity against most of our strains (only 3 strains were resistant).

High resistance to the most common anti-Enterococcal antimicrobials was reported in Iran ^(23, 24) Jordan ⁽²²⁾, Ethiopia ⁽²⁵⁾, and Sudan ⁽¹⁹⁾. The variation in isolation rate and resistance of enterococci may be due to differences in the study area, population, and protocol of antimicrobial uses. A high antimicrobial resistance

rate can make a real challenge for the treatment of Enterococcal infections. In this study, high-level resistance to Aminoglycosides, this is one of the traditionally most useful anti-Enterococcal.

Antimicrobial-resistant results in increased human illness, cost, and length of treatment. Patients being admitted for prolonged periods should be screened for VRE owing to this organism's capability of resistant gene transfer to other susceptible species within the hospital. There is a need for implementation of stringent infection control measures like rational use of antimicrobials especially restricting the use of Vancomycin to a minimum, proper containment, and effective treatment of VRE infections. Strict hand washing practices, education of the healthcare workers and other personnel involved in the patient management. These measures are to be strictly followed to bring down the mortality and morbidity associated with these nosocomial VRE infections

IV. CONCLUSION

There was a low-frequency rate of VRE and a wide range of other antimicrobial agents' resistance with an increase in multi-drug resistant strains of enterococci. Regular surveillance of antimicrobial susceptibilities should be done regularly and the risk factors should be determined.

V. Acknowledgment

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