High level aminoglycoside resistant enterococci in hospital-acquired urinary tract infections in Mansoura, Egypt

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Abstract

Introduction Resistance to different antimicrobial agents is increasing in enterococci and effective treatment represents a major health concern. The aim of this study was to determine the antimicrobial resistance patterns and the frequency of high level aminoglycoside resistance (HLAR) among enterococci.

Methods A total of 80 enterococcal isolates, (73 *Enterococcus faecalis*, 7 *Enterococcus faecium*) were collected from patients with hospital acquired urinary tract infections (UTI) at Mansoura University hospitals in Egypt. Antimicrobial susceptibility testing was performed via the disc diffusion method. PCR was used for identification of species and detection of aminoglycoside-modifying enzymes genes (AME).

Results All enterococcal isolates were sensitive to vancomycin and linezolid. Fifty-three isolates exhibited HLAR. Our results show that HLAR was mediated by the presence of multiple AMEs genes. The *aac(6')-Ie-aph(2)-Ia* gene was associated with *aph(3')-IIIa* and *ant(6)-Ia* gene in 69% of HLAR isolates.

Conclusion This study showed that enterococci isolated from hospital acquired UTI were resistant to multiple antibiotics. Furthermore, the frequency of high level gentamicin resistance (HLGR) was higher than high level of streptomycin resistance (HLSR). The most common AME genes were *aph(3')-IIIa* and *ant(6)-Ia* followed by *aac(6')-Ie-aph(2)-Ia*.

Keywords High level aminoglycoside resistance, enterococci, urinary tract infection.

Introduction

In recent decades, enterococci have become significant nosocomial pathogens with multiple-drug resistance mechanisms.¹ Urinary tract infection (UTI) is one of the most common type of infections generated by these organisms.² UTI

is a significant cause of morbidity and mortality among adults.³

The treatment of choice for serious enterococcal infections is an aminoglycoside in combination with a cell wall active agent.⁴ However, high-level aminoglycoside resistance (HLAR) is responsible for loss of synergy between agents active on the cell wall and aminoglycosides.⁵ In enterococci, HLAR is mediated by aminoglycoside-modifying enzymes (AMEs).⁶ There are three classes of AMEs: N-acetyltransferases (AAC), O-adenylyltransferases (ANT), O-phosphotransferases (APH).⁷

The rate of enterococci with HLAR and the distribution of AMEs vary across countries.⁸ Knowledge of the frequency of these resistance genes is important to inform the management of enterococcal infections. Little is known about the rate of HLAR enterococci in Egypt.

This study was conducted to determine the rate of HLAR and the distribution of AME genes in enterococci isolated from patients with hospital acquired UTI at Mansoura University Hospitals in Egypt.

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Methods

A cross-sectional study was conducted in Mansoura University Hospital (MUH), Mansoura, Egypt. Enterococci were isolated from urine samples collected from patients with hospital acquired (HA) UTI for a period of 2 years from August 2014 to July 2016 in MUH. Infections were considered as HA infections when new infection developed after 48 h of patient admission. The study was approved by the institutional research board (MS/16.11.19) at the Faculty of Medicine, Mansoura University.

Identification

Isolates were identified based on black colonies on bile esculin agar media, Gram staining, catalase test and bacterial growth in 6.5% NaCl.⁹ Multiplex PCR using specific *ddl E.faecalis* and *ddl E.faecium* genes was performed to identify both *Enterococcus faecalis* and *Enterococcus faecium* respectively.¹⁰

Antimicrobial susceptibility testing

The antimicrobial susceptibility tests were performed for enterococcal strains using the disc diffusion in accordance with Clinical and Laboratory Standards Institute (CLSI) criteria.¹¹ The following antibiotics were used: penicillin 10 U, ampicillin 10 µg, nitrofurantoin 10 µg, tetracycline 30 µg, ciprofloxacin 5 µg, vancomycin 5 µg, linezolid 10 µg and trimethoprim/sulfamethoxazole 25 µg. High level gentamicin (120 µg) and streptomycin (300 µg) discs (Mast Diagnostics, Merseyside, UK) were used to detect high level gentamicin resistance (HLGR) and high level streptomycin resistance (HLSR) respectively. Etests for gentamicin and streptomycin (Liofilchem, Roseto degli Abruzzi, Italy) were used for strains with zones of 7 to 9 mm to prove resistance or sensitivity.¹¹

PCR detection of AME

Six aminoglycoside resistance genes were detected in all strains via multiplex PCR conducted as previously reported⁵ with certain modifications: two multiplex PCR

amplifications were separately performed using 1) *aph(2)-Id*, *aph(3')-IIIa*, *ant(4')-Ia* primers 2) *aac(6')-Ie-aph(2'')-Ia*, *aph(2'')-Ib*, *aph(2'')-Ic* primers, separately. PCR was conducted in a Perkin-Elmer GeneAmp 2400 thermal cycler with an initial lysis step of 2 min at 94°C; 35 cycles of 40 s at 94°C, 60 s at 55°C, and 80 s at 72°C; and a final extension step of 5 min at 72°C.⁶ Another PCR was done for detection of *aac(6')-Ii*, *ant(6)-Ia* genes as previously described.⁸

PCR products were analyzed by electrophoresis in a 2% agarose gel that was stained with ethidium bromide. Primers used in this study and the product sizes of analyzed genes are listed in Table 1.

Statistical analysis

Data were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 16 (SPSS Inc., Chicago, IL, USA). Qualitative data are described as numbers and percentages. χ^2 test or Fisher's exact test were used for comparison between groups, as appropriate. Results with $p < 0.05$ were considered significant.

Results

During the study period, eighty enterococcal isolates were isolated from the urine samples of patients suffering from hospital acquired UTI. Seventy-three (91.25%) isolates were identified as *E. faecalis* and 7 (8.75%) as *E. faecium*. All enterococcal isolates were resistant to penicillin and ampicillin, but no isolates were resistant to vancomycin and linezolid. Antibiotic resistance to tetracycline and trimethoprim/sulfamethoxazole were 91.25% and 68.25% respectively. Seventy isolates (87.5%) of enterococcal isolates were multidrug resistant (MDR). There were no significant differences in antibiotic resistance patterns between *E. faecalis* and *E. faecium*. Antimicrobial resistance profiles of the enterococcal isolates are presented in Table 2.

Fifty-three (66.3%) enterococcal isolates (48 *E. faecalis* and 5 *E. faecium*) exhibited HLAR (both HLGR and HLSR) and 5 (6.3%) isolates exhibited HLGR alone. The *aph(3')-IIIa* and

Table 1. Specific primers used in this study

Gene	Primer sequence (5'-3')	Product size (bp)	Reference
<i>aac(6')-Ie-aph(2')-Ia</i>	CAGAGCCTTGGGAAGATGAAG CCTCGTGTAATTCATGTTCTGGC	348	5
<i>aph(2'')-Ib</i>	CTTGGACGCTGAGATATATGAGCAC GTTTGTAGCAATTCAGAAACACCCTT	867	5
<i>aph(2')-Ic</i>	CCACAATGATAATGACTCAGTTCCC CCACAGCTTCCGATAGCAAGAG	444	5
<i>aph(2')-Id</i>	GTGGTTTTTACAGGAATGCCATC CCCTCTTCATACCAATCCATATAACC	641	5
<i>aph(3')-IIIa</i>	GGCTAAAATGAGAATATCACCGG CTTTAAAAAATCATAACAGCTCGCG	523	5
<i>ant(4')-Ia</i>	CAAAGTCTAAATCGGTAGAAGCC GGAAAGTTGACCAGACATTACGAACT	294	5
<i>aac(6')-Ii</i>	TGGCCGGAAGAATATGGAGA GCATTTGGTAAGACACCTACG	410	8
<i>ant(6)-Ia</i>	CGGGAGAATGGGAGACTTTG CTGTGGCTCCACAATCTGAT	563	8
<i>ddl E. faecalis</i>	ATCAAGTACAGTTAGTCT ACGATTCAAAGCTAACTG	941	10
<i>ddl E. faecium</i>	TAGAGACATTGAATATGCC TCGAATGTGCTACAATC	550	10

Table 2. Antimicrobial resistance of *E. faecalis* and *E. faecium*

Antibiotic	<i>E. faecalis</i> (n=73)	<i>E. faecium</i> (n=7)	P-value	OR	(95%CI)
Ampicillin	73 (100%)	7 (100%)	N/A	N/A	N/A
Nitrofurantoin	34 (46.6%)	5 (71.4%)	0.285	2.868	(0.522-15.746)
Tetracycline	67 (91.8%)	6 (85.7%)	0.487	0.537	(0.055-5.231)
Ciprofloxacin	62 (84.9%)	4 (5.7%)	0.098	0.237	(0.046-1.206)
Trimethoprim/sulfamethoxazole	64 (87.7%)	5 (71.4%)	0.245	0.352	(0.059-2.089)
Linezolid	0 (0%)	0 (0%)	N/A	N/A	N/A
Vancomycin	0 (0%)	0 (0%)	N/A	N/A	N/A
HLG gentamicin	52 (71.2%)	6 (85.7%)	0.667	2.423	(0.275-21.367)
HLS streptomycin	48 (65.8%)	5 (71.4%)	1	1.302	(0.236-7.196)
Penicillin	73 (100%)	7 (100%)	N/A	N/A	N/A

Data are no. (%). HLG - high level gentamicin; HLS - high level streptomycin; N/A - not applicable.

aac(6')-Ie-aph(2')-Ia genes were identified in 53 and 52 HLR strains respectively, and *aph(2')-Id* was identified in 4 *E. faecium* isolates. All isolates with HLR carried the *ant(6)-Ia* gene. The *aph(2'')-Ib*, *aph(2')-Ic*, *ant(4')-Ia* and *aac(6')-Ii* were not detected in any of the HLR isolates. No PCR product was detected in any of the 22 non-HLR enterococci. Distribution of AME is summarized in Table 3.

Discussion

An increasing incidence of enterococci with high rates of resistance has been noticed in recent decades.¹² Several studies have showed that *E. faecalis* was the most common enterococcal isolate in clinical samples.¹³⁻¹⁵ These findings are consistent with the results of this study, in which 91.25% of enterococcal isolates were *E. faecalis*. However, another report stated that *E. faecium* infections are of increasing frequency.¹⁴

Table 3. Distribution of aminoglycoside-modifying genes in *E. faecalis* and *E. faecium*

Presence of aminoglycoside-modifying genes				<i>E. faecalis</i> (n=73)	<i>E. faecium</i> (n=7)
<i>aac</i> (6')- <i>Ie-aph</i> (2)- <i>Ia</i> (n=53)	<i>ant</i> (6)- <i>Ia</i> (n=53)	<i>aph</i> (3')- <i>IIIa</i> (n=54)	<i>aph</i> (2)- <i>Id</i> (n=4)		
+	+	+	-	39 (53.4%)	1 (14.3%)
+	+	-	-	4 (5.5%)	0 (0%)
+	+	+	+	0 (0%)	4 (57.1%)
-	+	+	-	5 (6.8%)	0 (0%)
+	-	+	-	4 (5.5%)	1 (14.3%)
-	-	-	-	21 (28.8%)	1 (14.3%)

In the present study, 87.5% of enterococci exhibited multidrug resistance (MDR). In previous studies, the rate of MDR varied from 45% to 100%.¹⁵⁻¹⁸ This high rate of MDR among enterococci may be due to the abuse of antibiotics and selective pressure. Although all of our isolates were sensitive to vancomycin, none of the isolates were sensitive to penicillin or ampicillin. This finding is in agreement with previously reported results.¹⁹⁻²¹ There was no significant difference in antimicrobial resistance between *E. faecalis* and *E. faecium*. In contrast, Celik et al. reported that antibiotic resistance percentages were higher in *E. faecium* than in *E. faecalis*.¹⁴

The rate of HLGR in enterococci varies from 1% to 89% in different regions.^{7,22,23} The most common gene associated with HLGR is *aac*(6')-*Ie-aph*(2)-*Ia*.^{5,7,24} In our study the rate of HLGR among enterococci was 72.5%. Both Del Campo and our group found the *aph*(3')-*IIIa* gene slightly more often than the *aac*(6')-*Ie-aph*(2)-*Ia* gene.²⁵

In this study, HLSR was detected at a lower rate than HLGR. Moreover, HLSR coexisted with HLGR. Prior publications have reported the same findings.^{16,21} In contrast, in other studies HLSR was more common than HLGR.^{25,26} In the current investigation, the *ant*(6)-*Ia* gene was detected in all HLSR isolates as previously reported.²⁵ However, this gene was detected at a lower rate in another study.²⁴

In this work, all isolates with HLAR had multiple AMEs genes. The *aac*(6')-*Ie-aph*(2)-*Ia* gene was associated with the *aph*(3')-*IIIa* and *ant*(6)-*Ia* gene in 69% of isolates with HLAR. This finding is consistent with previous reports.^{24,25}

The presence of many AME genes in the isolates indicate that neither gentamicin nor streptomycin can be used to achieve synergy with glycopeptides.²⁴

Conclusion

This study showed that enterococci isolated from hospital acquired UTI were resistant to multiple antibiotics. Furthermore, the frequency of HLGR was higher than that of HLSR. The most common AME genes were *aph*(3')-*IIIa* and *ant*(6)-*Ia* followed by *aac*(6')-*Ie-aph*(2)-*Ia*.

Authors' contributions statement: RE designed the study, carried out the microbiological tests, wrote the manuscript. RE, AM, GE shared in analysis and interpretation of data, drafting of the article and critical revision. All authors read and approved the final manuscript.

Conflicts of interest: All authors – none to declare.

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