Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2018 15(4): 4625-4629. **OPEN ACCESS**

Multiple antibiotic resistance of the emerging gut pathogen *Enterococcus*

Kamelia Osman[1]; Jihan Badr[2]; Maha El-hady[2] and Aalaa Saad[2]*

[1]Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, **Egypt**
[2]Animal Health Research, Institute, Dokki, **Egypt**.

***Correspondence:** aalaa.saad2019@gmail.com **Accepted: 0**5 Dec. 2018 **Published online:** 31Dec. 2018

Increasing multidrug resistance (MDR) in nosocomial *Enterococcus* strains from all over the world recently enhances the need for further investigation of enterococci, with special reference to their antibiotic resistance traits. The excessive use of antibiotics has led to consider them as important pollutants. Within this context, the identification of the phenotypic antibiotic resistant enterococcal strains in patients with UTI was implemented. The antibiotic susceptibility was determined against 13 antibiotics using the disc diffusion method. The enterococcal strains were 100% resistant to clindamycin, 10% resistant to linezolid.

Keywords: *Enterococcus faecalis*, *faecium,* antibiotic resistance

INTRODUCTION

In human infections, *E. faecalis* and *E. faecium* are the most prevailing species (>90%) (Goh et al., 2017). The majority of the enterococcal infections are endogeneous, but the crossed infection occurs mainly in hospitalized patients (Mukhopadhyay, 2018). *Enterococcus* in USA is the fourth most common cause of nosocomial infection and the third of bacteremia while in Europe, these infections are less and represent a 7.2% of the total (Padilla and Lobos, 2013).The emergence of enterococci as nosocomial and community-acquired pathogens was a consequence to their ability to develop high-level resistance to antimicrobials (Lee et al., 2018). The administration of antimicrobials in humans for the treatment and control of infections can select resistant strains (Catry, 2017). Antibiotic-resistant bacteria is a growing crisis worldwide. In the European Union, antibiotic resistance causes 25,000 deaths per year, in India, over 58,000 babies died in one year as a result of infection with resistant bacteria usually passed on from their mothers, in Thailand, antibiotic resistance causes 38,000+ deaths per year and in the United States, antibiotic resistance causes 23,000+ deaths per year (CDC, 2017). Strains with acquired resistance to vancomycin have been predominantly *E. faecium,* and has increased among hospital enterococcal isolates (Wardal et al., 2017; Sadowy et al., 2018). Within this context, the purpose of our study was to characterize a collection of enterococci isolated from patients with UTIs.

MATERIALS AND METHODS

Bacterial isolates and culture media

Enterococcal isolates were collected from Egyptian private hospitals during the period 2017. Identification of genus *Enterococcus* was done based on Gram staining, cultural characteristics and physiological and biochemical tests, namely, bile esculin hydrolysis, PYR hydrolysis, and growth in 6.5% sodium chloride and at pH 9.6. Further speciation was done by standard set of biochemical tests including arginine dihydrolase test, mannitol, sorbitol, sorbose, arabinose,

raffinose, lactose, sucrose (Sigma, USA), and pyruvate fermentation tests. The identity was further confirmed by cultivation on Brilliance UTI agar. This differential culture medium provides presumptive identification of several urinary tract pathogens. Unlike other species, *Enterococcus* species express β-glucosidase but not β-galactosidase or tryptophan deaminase. The β-glucosidase activity targets the chromogen, x-glucoside, and produces blue colonies. Isolates were subjected to species identification by using all the conventional biochemical methods described by Facklam et al., (2007). To confirm the isolates as Enterococci, definitive identification of all strains included in the study was performed targeting the Enterococcus genus-specific *Ent* gene polymerase chain reaction (fragment of 112 bp); F: TACTGACAAACCATTCATGATG and R: AACTTCGTCACCAACGCGAAC as previously described (Ke et al., 1999). Confirmed *Enterococcus* strains were streaked on Brain Heart agar slants and kept at 4 °C for the period of experimentation. Duplicate glycerol stocks of each isolate were stored at −80 °C.

Biofilm detection assay

Congo-red agar biofilm assay

The Congo red agar (Karimi et al., 2015) was used to assess the ability of isolates to form biofilm. The plates were incubated for 24 and 48 h at 35°C under aerobic and microaerophilic conditions. For each isolate the experiment was carried out in triplicate.

Antimicrobial susceptibility testing

All enterococci isolates were screened for susceptibility against 13 antibiotics by disc diffusion method and assigned as sensitive, intermediate and resistant according to the recommendations of The Clinical and Laboratory Standard Institute (CLSI, 2017). The antibiotics tested are considered among the most important drugs used in healthcare settings (WHO, 2017). This included: ampicillin, chloramphenicol, florfenicol, ciprofloxacin, norfloxacin, clindamycin, gentamycin, linezolid, erythromycin, oxyetracycline, doxycycline and vancomycin. *E. faecalis* ATCC 29212 and *E. faecium* ATCC 19434 were used as quality controls.

High level aminoglycoside resistance (HLAR)

Isolates that were resistant to gentamicin by disc diffusion test, were further examined for their resistance to higher concentrations of gentamicin (120 µg/mL) according to the recommendations of the CLSI (2017).

RESULTS

Identification and prevalence of *Enterococcus spp.*

A total of 20/30 (66.7%) *Enterococcus* species were identified as *E. faecium* 15/30 (50%) and *E. faecalis* 5/30 (16.7%).

Congo-red agar biofilm assay

E. faecium (6/15) and *E. faecalis* (2/3) recovered from urine isolates were 40% positive for biofilm formation by Congo red agar method for each.

Phenotypic Antimicrobial Resistance Patterns

The 20 enterococci were tested for their susceptibility to 13 antibiotics representing 10 different classes (Table 1). High level of antibiotic resistance was detected in all enterococci isolates evaluated. All 20 isolates were 100% resistant to clindamycin, oxytetracycline and gentamycin low level aminoglycoside resistance (LLAR)**,** while the lowest resistance was recorded against the clinically important antibiotic linezolid (4/20, 20%) a fortunate finding as linezolid is the last resort antibiotic in the clinical treatment with Gram-positive bacteria although resistance to ampicillin (85%) and vancomycin (90%), which are two important antibiotics medically used in human therapy against enterococcal infections, was clearly evident. The 20 isolates were seen to be resistant to four antibiotics (ampicillin, gentamycin (HLAR), florfenicol, chloramphenicol) at a rate of >70% and < 80%. High resistance was recorded against erythromycin (95%) followed by ciprofloxacin (90%), vancomycin (90%), norfloxacin (85%), doxycycline (80%), florfenicol (75%) and chloramphenicol (45%). Interestingly, only four out of 20 enterococci isolates (20%) were resistant to the clinically important antibiotic linezolid. The four enterococci isolates that were resistant to linezolid, were also resistant to 9-11 antibiotics, including vancomycin. In addition, 72.6% of enterococci isolates were resistant to ampicillin, an important antibiotic still in use for the treatment of enterococcal infection.

Table 1. Phenotypic antibiotic resistance profile for the enterococci isolated from human UTIs patients

Enterococcus species	ANTIBIOTICS												
	Ampicillin (10 µg)	**Clindamycin** (2 µg)	**Erythromycin** (15 µg)	**Oxytetracycline** (30 µg)	**Doxycycline** (30 µg)	**Gentamycin** (10 µg) (LLA)	**Gentamycin** (200 µg) **(HLA)**	**Ciprofloxacin** (5 µg)	**Norfloxacin** (10 µg)	**Linezolide** (30 µg)	**Florfenicol** (30 µg)	**Chloramphenicol** (30 µg)	**Vancomycin** (30 µg)
faecalis	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	1/5	4/5	2/5	4/5
faecium	12/15	15/15	14/15	15/15	11/15	15/15	11/15	13/15	12/15	3/15	11/15	7/15	14/15

LLAR, Low level aminoglycoside resistance; HLAR, High level aminoglycoside resistance

DISCUSSION

Strains with resistance to multiple antibiotic classes have emerged among *Enterococcus* spp.. In all samples we confirmed the presence of enterococci, with the predominance of *E. faecium* species endowed with MDR. The extent of *E. faecium* isolates has expanded to a great extent, because of the spread of resistance to the antimicrobials with special emphasis to vancomycin and ampicillin (Arias and Murray, 2012). The recorded combined resistance pattern of erythromycin and tetracycline observed in our isolates indicates that the use of tetracyclines may thus co-select for resistance to macrolides, which may be important as an alternative therapy for enterococcal infections (Cauwerts et al., 2007). The study of the epidemiology of enterococci with acquired antimicrobial resistance although interesting yet complex. Antibiotic resistance is not limited by geographical or biological borders (Ventola, 2015) and there are contrasting differences governed by the antibiotics used in the medical and veterinary practice, overuse, incorrectly prescribed antibiotics, extensive agricultural use, a stall in the development of new antibiotics by the pharmaceutical industry (Ventola, 2015) and in addition contrasts related with spread and colonization of people in various nations. Although *E. faecalis* is generally less drug resistant than *E. faecium*, we found that biofilm formation was more prevalent for *E. faecalis* than has been reported previously for *E. faecium* (Sandoe et al., 2003). The prevalence of *E. faecalis* biofilm observed in this study, 40%, was lower than the 60–90% prevalence rates reported previously in Europe (Toledo-Arana et al., 2001; Sandoe et al., 2003). Among the 5 *E. faecalis* isolates from urine with biofilm formation ability, 60% (3/5) of isolates were non-biofilm producers. Another study from Japan found in 352 *E. faecalis* strains from UTIs that all of them had the capacity to form biofilm, 37.5% (132/352) of isolates exhibited weak biofilm formation (Seno et al., 2005). In our endeavor, we detected high phenicol resistance among clinical isolates of enterococci in Egypt. Resistance levels for *E. faecalis* and *E. faecium* isolates were not comparable between geographical regions as geographical variation in resistance levels should be taken into consideration. In human, enterococci are not at all checked for this resistance in routine diagnostics since florfenicol is licensed exclusively for use in animals (WHO, 2012) and consequently, the number of enterococci isolates with resistance to florfenicol is obscure. Therefore, it should be emphasized that florfenicol may be useful when injectable antibiotic therapy is required in non-human primates (Cook et al., 2004).

CONCLUSION

This study reveals spreading of multidrug resistance *Enterococcus* spp. (*E. faecium* and E*. faecalis*) as UTI causing pathogen especially for most commonly used antibiotics in human treatment.

This finding is alarming since it suggests the possibility of transfer of these drug resistance ability to other Gram-positive bacteria as well as to enterococcal nonresistant species both in the GIT and environment.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

The author would thank all participants and their parents.

AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

REFERENCES

Arias CA, Murray BE (2012) The rise of the *Enterococcus*: beyond vancomycin resistance. Nature Rev Microbiol 10:266–278.

Catry B (2017) Antimicrobial policies in animals and human health. Archiv Public Health 75:62. http://doi.org/10.1186/s13690-017-0231-7

Cauwerts K, Decostere A, De Graef EM,

Haesebrouck F, Pasmans F (2007) High prevalence of tetracycline resistance in *Enterococcus* isolates from broilers carrying the erm(B) gene. Avian Pathol 36:395-99.

CDC (2017) Centers for Disease Control and Prevention. CDC Global Health, Global Health Infographics, Infographic: Antibiotic Resistance The Global Threat. 1600 Clifton Road Atlanta, GA 30329-4027 USA, Page last updated: July 10, 2017

CLSI (2017) Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.

Cook AL, St Claire M, Sams R (2004) Use of florfenicol in non-human primates. J Med Primatol33(3):127-133. https://doi.org/10.1111/j.1600-0684.2004.00063.x

Goel V, Kumar D, Kumar R, Mathur P, Singh S (2016) Community Acquired Enterococcal Urinary Tract Infections and Antibiotic Resistance Profile in North India. J Lab Physicians 8:50–54. http://doi.org/10.4103/0974-2727.176237

Goh HMS, Yong MHA, Chong KKL, Kline KA (2017) Model systems for the study of Enterococcal colonization and infection, Virulence 8: 1525-1562, DOI: 10.1080/21505594.2017.1279766

Karimi S, Ghafourian S, Taheri Kalani M, Azizi Jalilian F, Hemati S, Sadeghifard N (2015) Association Between Toxin-Antitoxin Systems and Biofilm Formation. Jundishapur J Microbiol 8:e14540. http://doi.org/10.5812/jjm.14540

Ke Danbing, François J. Picard, Francis Martineau, Christian Ménard, Paul H. Roy, Marc Ouellette, and Michel G. Bergeron. 1999 "Development of a PCR assay for rapid detection of enterococci." Journal of clinical microbiology 37,11 (): 3497-3503.

Lebreton F, Willems RJL, Gilmore MS (2014) *Enterococcus* Diversity, Origins in Nature, and Gut Colonization. 2014 Feb 2. In: Gilmore MS, Clewell DB, Ike Y, et al., editors. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection [Internet]. Boston: Massachusetts Eye and Ear Infirmary; 2014-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK190427/

Lee T, Pang S, Abraham S, Coombs GW (2018) Antimicrobial Resistant CC17 *Enterococcus faecium*: The Past, the Present and the Future. J Global Antimicrob Resis Available online 24 August 2018. DOI: https://doi.org/10.1016/j.jgar.2018.08.016

Mukhopadhyay C (2018) Infection control in intensive care units. Indian J Respir Care 7:14-21

Padilla C, Lobos O (2013) Virulence, bacterocin genes and antibacterial susceptibility in *Enterococcus faecalis* strains isolated from water wells for human consumption. Springer Plus 2, 43. http://doi.org/10.1186/2193-1801-2-43

Sadowy E, Gawryszewska I, Kuch A, Żabicka D, Hryniewicz W (2018) The changing epidemiology of *Van*B *Enterococcus faecium* in Poland. Europ J Clin Microbiol Infect Dis 37:927–936. https://doi.org/10.1007/s10096-018-3209-7

Sandoe JA, Witherden IR, Cove JH, Heritage J, Wilcox MH (2003) Correlation between enterococcal biofilm formation in vitro and medical-device-related infection potential in vivo. J Med Microbiol 52:547–550.

Seno Y, Kariyama R, Mitsuhata R, Monden K, Kumon H (2005) Clinical implications of biofilm formation by *Enterococcus faecalis* in the urinary tract. Acta Med Okayama 59:79–87.

Toledo-Arana A, Valle J, Solano C, Arrizubieta MJ, Cucarella C, Lamata M, Amorena B, Leiva J, Penades JR, Lasa I (2001) The enterococcal surface protein, Esp, is involved in *Enterococcus faecalis* biofilm formation. Appl Environ Microbiol 67:4538–4545.

Ventola CL (2015) The Antibiotic Resistance Crisis: Part 1: Causes and Threats. Pharm Therap 40:277–283.

Wardal E, Kuch A, Gawryszewska I, Żabicka D, Hryniewicz W, Sadowy E (2017) Diversity of plasmids and Tn1546-type transposons among *Van*A *Enterococcus faecium* in Poland. Eur J Clin Microbiol Infect Dis 36:313–328. DOI 10.1007/s10096-016-2804-8

WHO (2012) World Health Organization. Critically important antimicrobials for human medicine – 3rd rev. WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland

WHO (2017) World Health Organization. Critically important antimicrobials for human medicine – 5th rev. Geneva: World Health Organization; Licence: CC BY-NC-SA3.0 IGO.