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## **Emerging *Enterococcus gallinarum* infections and its antibiotic resistance in Karachi, Pakistan**

Rao Muhammad Abid Khan, Quratul-Ain and \*Zaid Ahmed Pirzada

Department of Microbiology, University of Karachi, Karachi 75270, Pakistan

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### **ABSTRACT**

This is the first report about the prevalence and the antibiotic resistance of the emerging pathogen *E. gallinarum* from Karachi, Pakistan. A total of 156 different clinical samples were collected from pathological laboratories and hospitals during April 2012 to 2014. *E. gallinarum* strains were identified by latex agglutination kit (Oxoid) and ABIS software online. Their resistance against seventeen antibiotics was determined by Kirby-Bauer disc diffusion method. Minimum inhibitory concentration (MIC) of vancomycin, macrolides (azithromycin, clarithromycin), clindamycin, ciprofloxacin and cefotaxime were determined by broth dilution method. In this study 51 *E. gallinarum* strains were isolated from 156 clinical samples. Maximum number of isolates was obtained from throat swab 29 (58%) followed by pus samples 16 (31%). *E. gallinarum* infections predominantly occurred during the cool season of the year. All *E. gallinarum* were resistant against streptomycin while sensitive to teicoplanin, imipenem, ampicillin and fosfomycin. More than half of the isolates were resistant against clindamycin, macrolides, penicillin, cefotaxime and ciprofloxacin, however the resistance against vancomycin and gentamycin was 40%. Over all the isolates from pus were more resistant than isolates from throat swabs. This report highlights a high level of resistance to a number of antibiotics making *E. gallinarum* an important emerging pathogen.

**Key words: Prevalence, bacteria, Pathogen, clinical, drug, streptococci**



### **INTRODUCTION**

Enterococci are the important nosocomial and community acquired pathogens responsible for significant morbidity and mortality worldwide [1,2]. It is reported to be the second most common cause of UTI's after *E. coli* and the third leading cause of bacteremia in hospital admitted patients. Beside this, enterococci are known to cause variety of invasive infections like oral, intra-abdominal and pelvic infections, bacterial endocarditis, neonatal sepsis, meningitis and root canal infections of teeth [3,4,5]. Debilitated, ill, immunocompromised, transplant and catheterized patients are at high risk of Enterococcal infections [5].

Medically important enterococcal species are *E. faecalis* and *E. faecium* which are responsible for 80-90% and 5-10% human Enterococcal infections respectively [5]. Other Enterococcal species especially the motile Enterococcus species; *E. gallinarum* and *E. casseliflavus* are the rare cause of clinically significant infections like bacteremia,

endocarditis and meningitis [6,7,8]. The clinical implication of motile Enterococcal species is still indeterminate, however; both of these species constitute 1-2% of all human Enterococcal infections [6,7,9]. *E. gallinarum* and *E. casseliflavus* have been reported to cause a range of invasive infections in humans especially in immunocompromised, chronically ill or debilitated individuals [6,7,8]. Motile Enterococcal species have also been reported to colonize the intestinal gut of hospitalized and healthy individuals [6,7].

Enterococcal infections are usually treated with  $\beta$ -lactams in combination with aminoglycosides while glycopeptides especially vancomycin is reserved as a last therapeutic choice. The emergence of resistance among Enterococci against various antibiotics especially  $\beta$ -lactams, aminoglycosides and glycopeptides limits the therapeutic options. Enterococci may be intrinsically or extrinsically resistant to different antibiotics and acquire resistance through mutations in chromosome, chromosomal exchange,

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\*Corresponding Author Address: Zaid Ahmed Pirzada, PhD, Assistant Professor, Department of Microbiology, University of Karachi, Karachi 75270, Pakistan; E-mail: [zaidahmed@uok.edu.pk](mailto:zaidahmed@uok.edu.pk)

conjugation, transposon or plasmids [10,11]. The antibiotic resistance pattern varies according to geographical location and from time to time as they can transfer antibiotic resistance genes to other bacteria. Epidemiological studies also suggest Enterococci being an important reservoir of antibiotic resistance genes, responsible for transmission among different bacterial species. There is dire need to use the antibiotics properly for Enterococcal infections as choices for their treatment are becoming limited.

The objective of current study was to evaluate the prevalence and antibiotic resistance of *E. gallinarum* among various clinical samples in Karachi, Pakistan.

## MATERIALS AND METHODS

This study was performed in the Department of Microbiology, University of Karachi, Pakistan from April 2012 to 2014. Blood agar plates containing 5% blood were used and altogether 156 clinical samples of throat swab, pus, blood, body fluids, sputum and urethral swab were collected from different pathological laboratories, tertiary care hospitals and medical centres. Strains were classified as Group D streptococci (GDS) by latex agglutination reaction (Oxoid diagnostic reagent) and were confirmed as *E. gallinarum* by performing various biochemical identification tests *i.e.* bile esculin hydrolysis, 6.5% NaCl, growth at 10°C and 45°C [12], fermentation of various sugars (Arabinose, Glucose, Lactose, Mannitol, Raffinose, Sorbitol, Sucrose and Trehalose) and motility testing etc. with the help of ABIS (Advanced Bacterial Identification Software) online ([http://www.tgw1916.net/bacteria\\_logare.html](http://www.tgw1916.net/bacteria_logare.html))

The antibiotic resistance pattern was performed using blood agar plates by Kirby-Bauer disc diffusion method in triplicates and average value was taken for interpretation against following groups of antibiotics *i.e.* Penicillin [penicillin G, ampicillin, amoxicillin/clavulanic acid], Macrolides [azithromycin, clarithromycin, erythromycin], Lincosamide [clindamycin], Quinolone [ciprofloxacin], Cephalosporin [cefotaxime], Glycopeptides [vancomycin, teicoplanin], Aminoglycoside [streptomycin, gentamycin], Carbapenem [imipenem], Oxazolidone [linezolid], Tetracycline and Fosfomycin. CLSI guidelines were used for results interpretation.

Minimum inhibitory concentration (MIC) to check the level of resistance was determined by broth dilution technique. MIC<sub>50</sub> and MIC<sub>90</sub> were calculated.

## RESULTS

Fifty one Enterococci were isolated from a total of 156 clinical specimens with the prevalence rate of 33%. Majority of the Enterococci were isolated from throat swabs 29 (58%) and pus 16 (31%) followed by blood 2 (4%), body fluids 2 (4%), sputum 1 (2%) and urethral swab 1 (2%). (Figure1). Majority of the *E. gallinarum* species were isolated during cool season (Table-1).

All *E. gallinarum* strains were resistant to streptomycin while a higher level of resistance was seen against clindamycin (96%), azithromycin (75%), penicillin (73%), cefotaxime (67%), clarithromycin (51%) and erythromycin (45%), however; 40% of *E. gallinarum* were resistant against vancomycin and gentamycin. The lowest resistance was observed against linezolid (2%), Co-amoxiclavate (4%) and tetracycline (7%). Importantly, in this study we found all *E. gallinarum* strains sensitive to teicoplanin, imipenem, ampicillin and fosfomycin. (Figure2)

Interestingly, in this study we found that *E. gallinarum* isolated from throat swabs were less resistant than *E. gallinarum* from pus, in contrast *E. gallinarum* strains isolated from throat swab specimens were significantly more resistant to glycopeptides and aminoglycosides than from pus specimens (Table-2).

MIC results show very high level of resistance among *E. gallinarum* against macrolides (512-1024 µg/ml), lincosamides (1024 µg/ml), quinolones (64 µg/ml) and cephalosporins (64 µg/ml) however, fourfold resistance was observed against vancomycin (Table-3)

## DISCUSSION

Motile enterococci are infrequently encountered in clinical specimens and represent 1-2% of all enterococcal infections, so the clinical experience with these strains is limited. Literature review reveals that motile enterococci cause a range of invasive infections in humans especially in immunocompromised, chronically ill or debilitated individuals or the patients with underlying medical problems such as diabetes mellitus, renal failure or chronic osteomyelitis. The low prevalence of motile Enterococci may be correlated with difficulties in identifying the Enterococcal species. Studies also reveal that motile Enterococci may colonize the intestinal tracts of both hospitalized and non-hospitalized individuals. Motile Enterococci usually exhibit intrinsic low level of resistance to vancomycin ranging from 2-32 µg/ml

which is conferred by the chromosomal *vanCI* gene.

Enterococcal infections are classically treated with  $\beta$ -lactam drugs (penicillin, amoxicillin) in combination with aminoglycosides (gentamicin, streptomycin) [13,14]. Failure of treatment with these antibiotics, glycopeptides especially vancomycin remains the drug of choice. However, there are many reports of high-level of resistance to  $\beta$ -lactams, aminoglycosides and glycopeptides [13,15,16]. Patients which are infected with Vancomycin resistant enterococci (VRE) along with HLAR (high level of aminoglycoside resistance) are extremely difficult to treat and are great challenge to the physicians and microbiologists. Enterococci also have intrinsic mechanism of resistance to many groups of antibiotics *i.e.* aminoglycosides, quinolones and cephalosporins [17].

Several genes *i.e.* *van A, B, C, D* and *E* have been linked to vancomycin resistance [5]. Among these genes *van A* is frequently isolated and is reported to be present in *Tn1546* transposon and can be transferred to other bacteria [17]. In these patients the last choice is linezolid and teicoplanin while the UTI's are treated with nitrofurantoin [13,14,18]. VRE may cause variety of infections relating with high mortality rate especially in endocarditis and bacteremia cases. VRE, resistant to other antibiotics concomitantly worsen the condition and resulting in the treatment failure and death.

In present study the highest number of isolates were collected from throat swabs 29 (58%) followed by pus 16 (31%). Very limited data is available about the prevalence of motile enterococci however few studies report the isolation of *E. gallinarum* from blood or CSF specimens in patients with underlying medical problems.

In our study highest rate of resistance was observed against clindamycin *i.e.* (90%) followed by

azithromycin (75%), penicillin (73%), cefotaxime (67%), ciprofloxacin (63%), clarithromycin (51%) and erythromycin (45%). The current report from Pakistan shows very high level of resistance compared to studies conducted in other countries, while the MIC of vancomycin ranged from 2-4 $\mu$ g/ml which is in accordance with findings of previous studies [7].

Motile enterococcal species *E. gallinarum* and *E. casseliflavus* are the infrequent cause of serious invasive infections in humans and together these species cause 1-2% of all enterococcal infections. *E. gallinarum* is intrinsically resistant to low levels of vancomycin. Antibiotic treatment failure has become a common issue in the treatment of enterococcal infections. In past *E. gallinarum* didnot show virulence against human but now it is also encountered as pathogenic for human and isolated as the causative agent of different human infections like endocarditis, ocular trauma, endophthalmitis, urinary tract infections, meningitis, serious invasive infections and bacteremia in immune-suppressed patients [6,7,8].

In our study the isolates collected from pus were more resistant than collected from throat swabs, might be because usually wounds are infected with two or more types of bacteria and are treated with combined therapy or with topical agents. This rough use of antibiotics may have increased their drug resistance. Furthermore, they may also acquire resistance genes from each other or from the environment.

This study questions the concept that *E. faecalis* or *E. faecium* are the prominent Enterococcus species among human infections. Correct, immediate diagnosis and restricted use of antibiotics is therefore recommended. It is important that studies in other countries should also be carried out to get better insight about *E. gallinarum* prevalence and resistance profile.

<b>Table-1. Seasonal prevalence of <i>E. gallinarum</i></b>		
<b>Season Name</b>	<b>Range of month</b>	<b>GDS n (%)</b>
<b>Hot season</b>	<b>March to June</b>	<b>18 (35%)</b>
<b>Rainy season</b>	<b>July to October</b>	<b>12 (24%)</b>
<b>Cool season</b>	<b>November to February</b>	<b>21 (41%)</b>
<b>Total</b>		<b>51</b>

Source	Macrolides			Lincosamide	Quinolone	Penicillin		Cephalosporin	Glycopeptide	Aminoglycoside	Tetracycline	Oxazolidone
	Azithromycin	Clarithromycin	Erythromycin	Clindamycin	Ciprofloxacin	Penicillin	Co-amoxiclavate	Cefotaxime	Vancomycin	Gentamycin	Tetracycline	Linezolid
<b>Throat swab</b>	R	R	R	R	R	R	R	R	R	R	R	R
<b>%</b>	72%	45%	45%	93%	62%	72%	3%	62%	60%	64%	8%	4%
<b>Pus</b>	R	R	R	R	R	R	R	R	R	R	R	R
<b>%</b>	87%	62%	56%	94%	69%	81%	6%	81%	13%	7%	7%	0%

Table. 2. Comparison of resistance among *E. gallinarum* isolated from throat swabs and pus

Antibiotic Group	Antibiotics (No. of Strains)	Breakpoints (Sensitive/Resistance)	Range (µg/ml)	MIC (µg/ml)	MIC <sub>50</sub> (µg/ml)	MIC <sub>90</sub> (µg/ml)	Level of resistance (No. of times)
Macrolides	Clarithromycin (23)	≤0.25/≥1	0.5- 1024	>1024	32	512	1024
	Azithromycin (35)	≤0.5/≥2	0.5- 1024	>1024	512	1024	512
Lincosamide	Clindamycin (43)	≤0.25/≥1	0.5- 1024	>1024	512	1024	1024
Quinolone	Ciprofloxacin (29)	≤1/≥4	0.5 - 512	256	16	64	64
Cephalosporin	Cefotaxime (31)	≤0.5/≥1	0.5- 1024	64	8	16	64
Glycopeptide	Vancomycin (18)	≤1/>1	0.5-256	4	2	4	4

MIC break points are according to CLSI

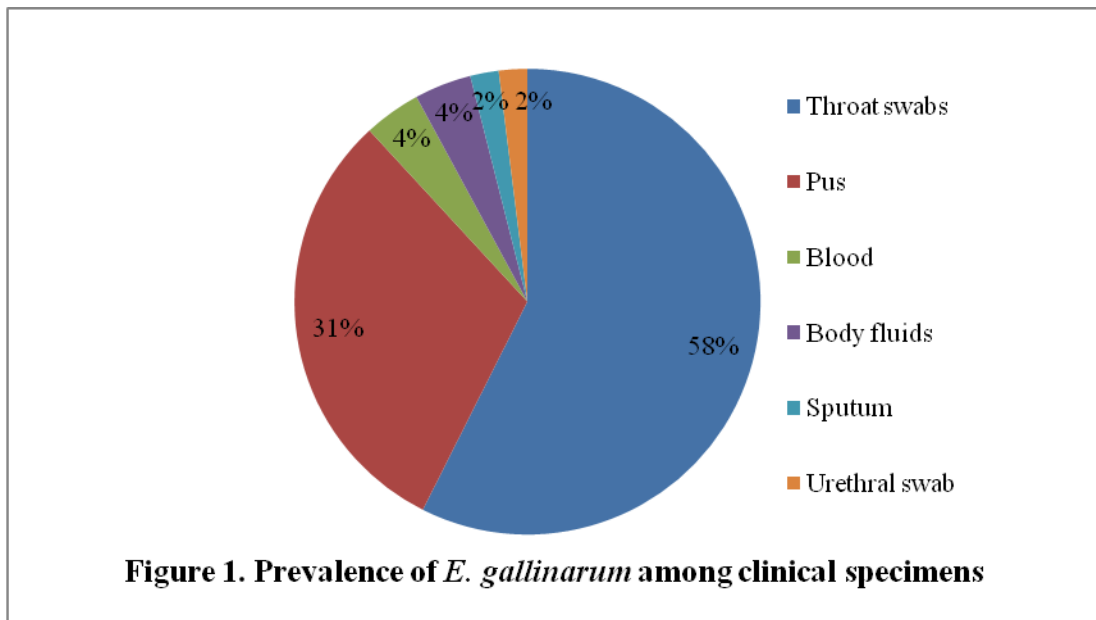
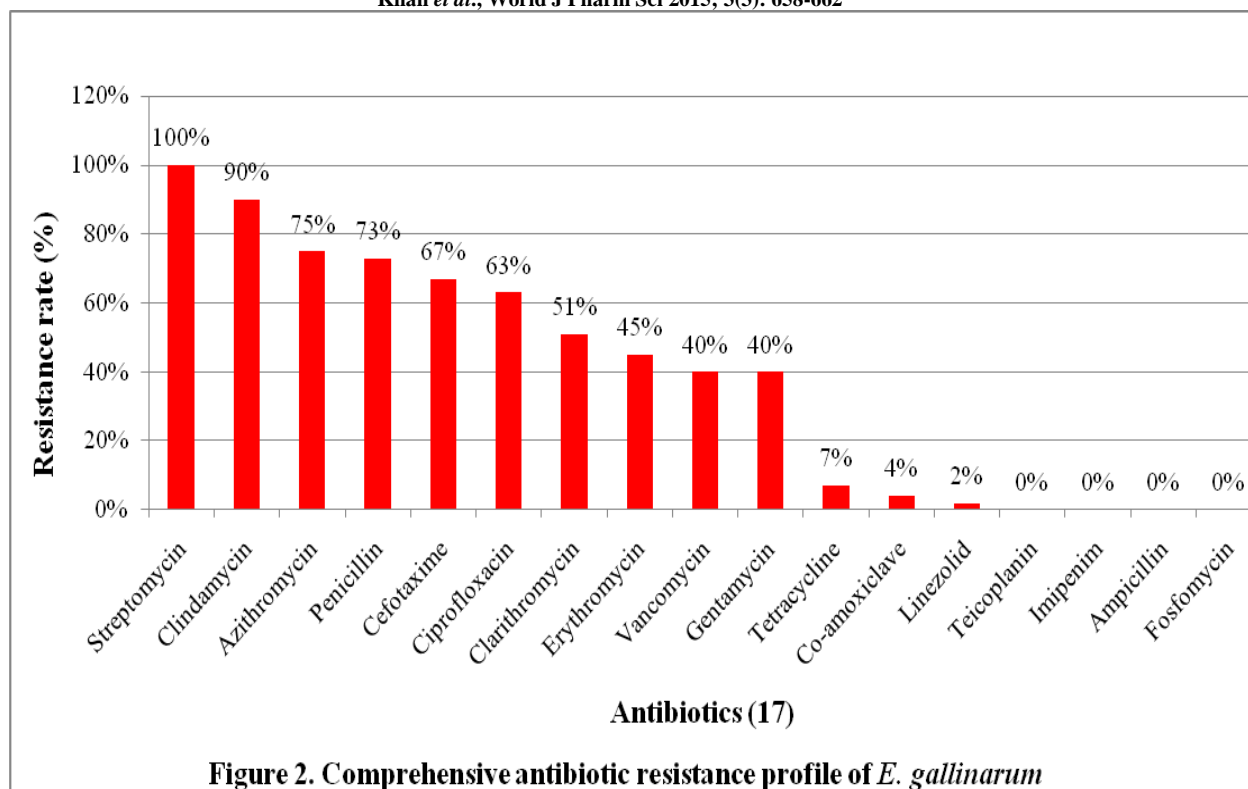


Figure 1. Prevalence of *E. gallinarum* among clinical specimens



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