ORIGINAL ARTICLE

Linezolid Resistance in Tanta University Hospitals: a Cross-sectional Study

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ABSTRACT

Background: *Linezolid abuse has led to the emergence of linezolid-resistant strains. cfr, optrA, and poxtA are among the most important resistance-determining genes.* ***Objective:*** *To detect the antibiotic susceptibility patterns of community-acquired (CA) and hospital-acquired (HA) Gram-positive isolates in Tanta University Hospitals, and screen linezolid-resistant isolates for cfr, optrA, and poxtA genes.* ***Methodology:*** *Subjects recruited in this study were 168, and 232 patients with CA and HA infections, respectively. The specimens were collected from various sources, including skin and subcutaneous tissue infections, respiratory tract infections, empyema, peritonitis, urinary tract infections and bacteremia. Assessment of antibiotic susceptibility profiles of the Gram-positive isolates against cefoxitin, ciprofloxacin, erythromycin, gentamicin, linezolid, oxacillin, penicillin, quinupristin/dalfopristin, tigecycline, and vancomycin was done by disc diffusion method. Then, conventional polymerase chain reaction for cfr, optrA, and poxtA was done in case of linezolid-resistant isolates.* ***Results****: The number of Gram-positive isolates were 73 from CA infections and 73 from HA infections. Two HA isolates were linezolid-resistant; both were cfr-positive, optrA- and poxtA-negative.* ***Conclusions****: Linezolid resistance was detected only in HA Gram-positive isolates at a percentage of 2.74%. Only cfr gene was detected in linezolid-resistant isolates.*

INTRODUCTION

The emergence of antibiotic resistance around the world, mainly in developing countries, represents a major threat to humanity as it occurs at an extremely fast pace[1]. This makes the expectation of the post-antibiotic era not far from imagination[1].

The unregulated use and abuse of antibiotics makes the issue even more difficult, with consequent appearance and wide spreading of MDR (multidrug-resistant) and XDR (Extensively drug-resistant) bacterial strains[2]. For treatment of patients infected with these strains, physicians prescribe last resort antibiotics, which have potent antimicrobial effects with low resistance rates[3]. Linezolid is one of the antibiotics of last resort[4]. It is the most important drug of the oxazolidinones family of antibiotics[5]. It was approved by the US Food and Drug Administration (FDA) in 2000[5]. It is highly efficient against MDR and XDR Gram-positive strains, with relatively low resistance rates[4,5]. In addition, its pharmacokinetic profile is excellent with efficient oral absorption, distribution and tissue penetration, which makes it ideal for the management of many diseases[5].

Linezolid overuse and extensive use are widely observed in clinical practices in both developed and developing countries due its excellent pharmacodynamic and pharmacokinetic properties[6, 7]. This played a major role in the emergence of Gram-positive linezolid-resistant strains[8]. The mechanism by which bacteria develop resistance to linezolid can be due to either chromosomal mutation or acquisition of mobile genetic elements carrying certain linezolid-resistance genes such as *cfr*, *optrA* and *poxtA*[9, 10].

In order to wisely deal with this issue, accurate and proper evaluation of the rate and the size of linezolid resistance should be estimated. The objective of this study came in this context. The current study aimed to precisely estimate the antibiotic susceptibility patterns of the Gram-positive organisms involved in community-acquired (CA) and hospital-acquired (HA) infections in Tanta University Hospitals, including phenotypic detection of resistance against linezolid, followed by detection of *cfr*, *optrA*, and *poxtA* genes in linezolid-resistant isolates.

METHODOLOGY

Subjects: This study was carried out at the Medical Microbiology and Immunology Department, Faculty of Medicine, Tanta University. It was done throughout the period from December 2019 to November 2020 and included 400 patients admitted to different wards and visiting different outpatient clinics of Tanta University Hospitals. Participants of the current study provided written informed consents. Ethical approval for the current study was granted by the ethics and research committee, Faculty of Medicine, Tanta University (No: 33423/10/19).

***Inclusion criteria for cases*:** Patients older than 18 years old, with clinical diagnosis of pathogenic bacterial infections.

***Exclusion criteria for cases*:** Patients with good response to antibiotic therapy.

Specimens: The specimens were collected according to the clinical condition. They included Sputum, bronchoalveolar lavage (BAL), urine, wound swabs, pus, blood, and pleural and peritoneal fluids' aspirates

Isolation and identification of the infecting organisms:

All specimens were cultured on blood, nutrient, MacConkey, and Sabouraud agar plates. All agar plates were incubated for 24- 48 hours at 37°C. All culture media were manufactured by Oxoid, UK. The organisms were identified by conventional microbiological methods[11].

Antibiotic sensitivity testing:

The antibiogram profiles of the Gram-positive isolates were assessed by modified Kirby Bauer disc diffusion method, except for determining the susceptibility of staphylococcal isolates to vancomycin that was tested using E-test according to the Clinical and Laboratory Standard Institute (CLSI) guidelines[12]. Gram-positive isolates were tested against cefoxitin (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), linezolid (30 µg), oxacillin (1 µg), penicillin (10 µg), quinupristin/dalfopristin (15 µg), tigecycline (15 µg), and vancomycin (30 µg). All of them were manufactured by Oxoid, UK. In case of *Staphylococci*, vancomycin MIC test strips (0.016-256 µg/ml) (Liofilchem, Italy) were used.

Detection of *cfr*, *optrA*, and *poxtA* genes:

Deoxyribonucleic acid (DNA) was extracted by Wizard® Genomic DNA Purification Kit (Promega, US), according to the manufacturer's protocol. *cfr*, *optrA*, and *poxta* genes amplifications were performed by conventional polymerase chain reaction (PCR) using a thermal cycler (Gene Amp, PCR system 9700), GoTaq® long PCR master and the primers shown in table 1. The PCR amplification conditions for *cfr* gene were as follow: initial denaturation at 94°C for 1 min, followed by 34 cycles of denaturation at 94° C for 1 min; annealing at 48° C for 2 min; extension at 72° C for 3 min and final extension at 72° C for 7 min. The PCR conditions for *optrA* gene were the same except for the annealing temperature which was at 55° C. For *poxtA* gene, PCR conditions were initial denaturation at 95°C for 2 min, followed by 25 cycles of denaturation at 95° C for 15 s; annealing at 53° C for 15 s; extension at 68° C for 90 s and final extension at 68° C for 5 min.

Amplified PCR products were detected electrophoresis on 1% agarose gel containing 1 µg/ml ethidium bromide and were visualized under ultraviolet light.

Table 1. Primers used for detection of *cfr*, *optrA*, and *poxta* genes and expected amplicon sizes.

Gene	Primer	Nucleotide sequence	Amplicon size	Reference
cfr	*cfr*-forward	TGA AGT ATA AAG CAG GTT GGG AGT CA	746 bp	13
	cfr-reverse	ACC ATA TAA TTG ACC ACA AGC AGC		
optrA	*optrA*-forward	AGG TGG TCA GCG AAC TAA	1,395 bp	14
	optrA-reverse	ATC AAC TGT TCC CAT TCA		
poxtA	*poxtA*-forward	TCA GAG CCG TAC TGA GCA AC	167 bp	15
	poxtA-reverse	CGT TTC TGG GTC AAG GTG GT		

bp: base-pair.

Statistical analysis:

Statistical analysis of data was done using the Statistical Package for the Social Sciences (SPSS), version 20 (Armonk, NY: IBM Corp). The quantitative variables were expressed as means and standard deviations. The categorical variables were presented by the numbers and the percentages. Student's t-test was used to evaluate the statistical significance of the differences in case of quantitative variables In case of qualitative variables, chi-square test was used. P-values less than 0.05 were considered statistically significant.

RESULTS

Distribution of cases:

The current study included 400 patients; 232 of them had HA infections, whereas 168 patients had CA infections. Demographic data of the patients is illustrated in table 2. There was no statistically significant difference between the two study groups concerning age and sex.

Table 2: Demographic data of the two study groups.

		CA infections (n=168)	HA infections (n=232)	Total (n=400)	P-Value
Age	**Range**	18 - 82	18 - 89	18 - 89	0.183
	Mean ±SD	43.41 ± 17.43	45.72 ± 16.41	44.73 ± 16.85	
Sex	**Male**	72 (42.86%)	122 (52.59%)	194 (48.5%)	0.055
	Female	96 (57.14%)	110 (47.41%)	206 (51.5%)	

CA: community-acquired; HA: hospital-acquired.

Respiratory specimens, including sputum and BAL, were the most frequent among the two study groups, followed by urinary specimens. Three types of specimens, including blood and ascitic and plural fluids, were exclusively collected from HA group. Details of the numbers of collected specimens from the two study groups are shown in table 3. The types of growth yielded from specimens of the two groups is shown in figure 1.

Table 3: The source of clinical specimens collected from the two study groups.

Clinical infection	Specimen	CA infections n=168	HA infections n=232	Total n=400	P-value
Skin and subcutaneous tissue infections	Wound swabs	14 (7.74%)	15 (6.90%)	29 (7.25%)	0.606
	Bed sore swab	0 (0%)	7 (3.02%)	7 (1.75%)	0.059
	Infected ulcers swabs	2 (1.19%)	0 (0%)	2 (0.5%)	0.343
	Diabetic foot swabs	18 (10.71%)	4 (1.72%)	22 (5.5%)	<0.001*
	Pus (abscesses and sinuses)	21 (12.5%)	3(1.29%)	24 (6%)	<0.001*
Respiratory tract infections	Sputum	46 (27.38%)	67 (28.88%)	113 (28.25%)	0.829
	BAL	0 (0%)	26 (11.21%)	26 (6.5%)	<0.001*
Empyema	Pleural fluid	0 (0%)	7 (3.02%)	7 (1.75%)	0.059
Peritonitis	Ascitic fluid	0 (0%)	7 (3.02%)	7 (1.75%)	0.059
Urinary tract infection	Urine	67 (39.88%)	47 (20.26%)	114 (28.5%)	<0.001*
Bacteremia	Blood	0 (0%)	49 (21.12%)	49(12.25%)	<0.001*

CA: community-acquired; HA: hospital-acquired; BAL: bronchoalveolar lavage. *: Significant at P-value < 0.05.

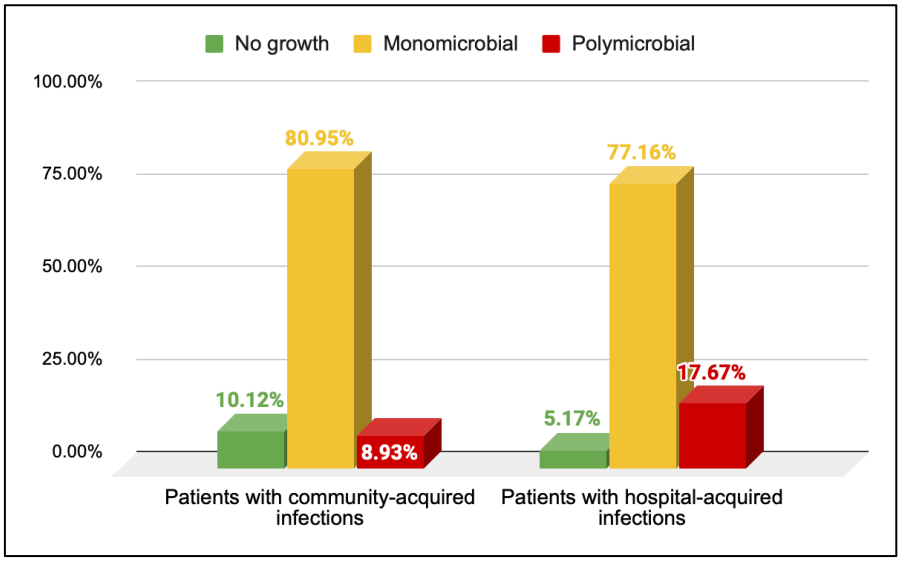

Figure 1: The type of growth of specimens of the two groups.

Types of isolates:

Gram-negative isolates were the most frequent among the two study groups. Fungal isolates were much more frequent in the HA group. The number of Gram-positive isolates was the same in the two study groups, being 73 in each. However, the percentage of Gram-positive isolates was much higher in the CA group than the HA group. *Staphylococcus aureus* (*S. aureus*) came in the first rank among all Gram-positive bacteria in the two groups. Detailed numbers and percentages are shown in table 4.

Table 4: Types of microbial isolates from the two study groups.

Isolated organisms	CA isolates (n=177)	HA isolates (n=283)	Total (n=460)	P-value
Fungi	1 (0.56%)	29 (10.25%)	30 (6.52%)	< 0.001*
Gram-negative	103 (58.19%)	181 (63.96%)	284 (61.74%)	0.255
Gram-Positive	73 (41.24%)	73 (25.79%)	146 (31.74%)	< 0.001*
● ***S. aureus***	50 (68.49%)	39 (53.42%)	89 (60.96%)	0.124
● **CoNS**	14 (19.18%)	15 (20.55%)	29 (19.86%)	
● ***Enterococci***	8 (10.96%)	18 (24.66%)	26 (17.81%)	
● ***Pneumococci***	0 (0%)	1(1.37%)	1 (0.68%)	
● ***S. pyogenes***	1(1.37%)	0 (0%)	1 (0.68%)	

CA: community-acquired; HA: hospital-acquired; *S. aureus*: *Staphylococcus aureus*; CoNS: coagulase-negative *Staphylococci*; *S.pyogenes*: *Streptococcus pyogenes.* *: Significant at P-value < 0.05.

Antibiotic susceptibility profile of the Gram-positive isolates:

The antibiotics showing best susceptibility profiles and lowest resistance rates were linezolid, tigecycline, and vancomycin. All of CA isolates were susceptible to all of them, while 2.74% of the HA isolates were resistant to each of the three drugs. The resistance rates of cefoxitin in the two groups were more or less similar. Conversely, the resistance percentages of gentamicin, ciprofloxacin and erythromycin were somewhat higher in HA isolates. Detailed data is shown in table 5.

There was extreme prevalence of MDR bacteria among the two study groups with a final percentage of 69.18%. In the CA group: 48 (65.75%) isolates were MDR and 25 (34.25%) isolates were non-MDR. In the HA group: 53 (72.6%) cases were MDR and 20 (27.4%) cases were non-MDR. The difference between the two study groups was not statistically significant.

Table 5: Antibiotic susceptibility profile of the Gram-positive isolates.

Antibiotic	CA cases (n = 73)			HA cases (n = 73)			P-value
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant	
Linezolid	73 (100%)	0 (0%)	0 (0%)	71 (97.26%)	0 (0%)	2 (2.74%)	0.154
Vancomycin	73 (100%)	0 (0%)	0 (0%)	71 (97.26%)	0 (0%)	2 (2.74%)	0.154
Tigecycline	71 (98.61%)	1 (1.39%)	0 (0%)	68 (93.15%)	3 (4.11%)	2 (2.74%)	0.217
Quinupristin /Dalfopristin	52 (88.14%)	6 (10.17%)	1 (1.69%)	41 (70.69%)	11 (18.97%)	6 (10.34%)	0.042*
Ciprofloxacin	39 (54.17%)	8 (11.11%)	25 (34.72%)	29 (40.27%)	8 (11.11%)	35 (48.61%)	0.208
Erythromycin	25 (34.25%)	21 (28.77%)	27 (36.99%)	18 (24.66%)	18 (24.66%)	37 (50.68%)	0.231
Gentamicin	20 (37.04%)	3 (5.56%)	31 (57.40%)	20 (31.25%)	1 (1.56%)	43 (67.19%)	0.347
Cefoxitin	17 (26.56%)	0 (0%)	47 (73.44%)	10 (18.52%)	2 (3.7%)	42 (77.78%)	0.195
Oxacillin	3 (21.43%)	0 (0%)	11 (78.57%)	2 (13.33%)	0 (0%)	13 (86.67%)	0.846
Penicillin	3 (4.11%)	0 (0%)	70 (95.89%)	3 (4.11%)	0 (0%)	70 (95.89%)	1

CA: community-acquired; HA: hospital-acquired; *: Significant at P-value < 0.05.

Clinico-microbiological profile of the 2 patients having linezolid-resistant isolates:

The first patient was a female (22 years old), who was admitted to hospital as a case of pancytopenia for investigations which yielded a final diagnosis of acute leukemia. Three days later, she developed a fever of unknown cause. Blood culture yielded a coagulase negative *Staphylococci* (CoNS) isolate, with subsequent diagnosis of septicemia. The antibiotic susceptibility profile of the isolate showed resistance to linezolid.

Medical history revealed that the patient suffered from multiple attacks of infections. During one of them, she had suffered from a high fever and sore throat. She visited a private clinic where the otorhinolaryngologist prescribed azithromycin. However, the clinical symptoms got worse, so he prescribed empirical therapy of linezolid with no previous culture and sensitivity.

The second patient was a diabetic and hypertensive 67-year-old male who had below-knee amputation 2 weeks before the collection of the specimen. Six days later, the amputation stump developed a surgical wound infection. Surgical wound swab collected from the amputation stump yielded a MDR *S.aureus* that was resistant to linezolid.

Detection of *cfr*, *optrA* and *poxtA* genes by PCR:

Both of the linezolid-resistant strains were positive for *cfr* gene. However, both of them were negative for *optrA* and *poxtA* genes as shown in figure 2.

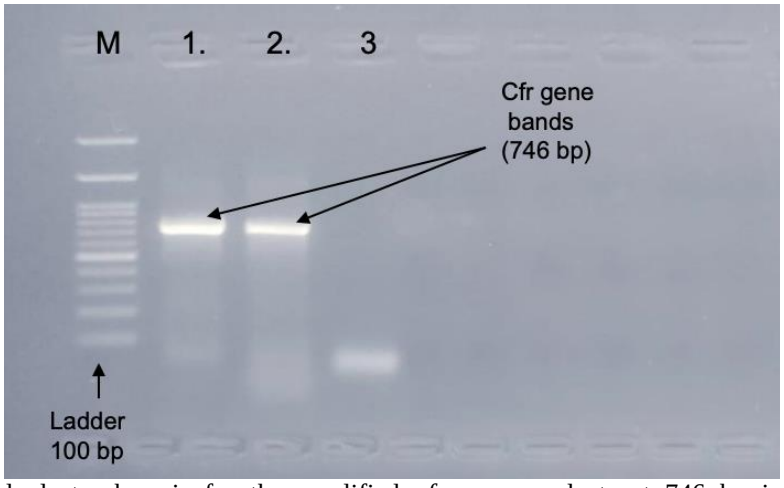

Figure 2: Agarose gel electrophoresis for the amplified *cfr* gene products at 746 bp in the linezolid-resistant *Staphylococcus aureus* isolate (Lane 1) and in the linezolid-resistant CoNS isolate (Lane 2). Lane 3 shows the negative control strain (Linezolid-susceptible *Staphylococcus aureus* ATCC 29213). Lane M shows a 100-bp DNA ladder

DISCUSSION

Linezolid is one of the last resort drugs against MDR Gram-positive pathogens because it has a unique mechanism of action and relatively low rates of resistance around the world[4]. Additionally, its pharmacokinetic properties are excellent, with nearly 100% bioavailability after oral administration[5]. Moreover, its safety profile is very good[5]. For all these factors, extensive use and overuse of linezolid are widely observed, which contributed to the emergence of linezolid-resistant strains[16].

This study was the first to investigate for the presence of linezolid-resistance genes *cfr*, *optrA* and *poxtA* in Tanta University Hospitals, and the first study in Egypt to search for *poxtA*-mediated resistance.

In the current study, the percentages of Gram-negative isolates were 58.19% and 63.96% of CA and HA isolates, respectively, being higher than Gram-positive isolates in both study groups. On the other hand, the percentage of Gram-positive infections was much higher in CA cases (41.24%) than HA cases (25.79%); the difference between the two groups was statistically significant ($P< 0.001$). This study's results came in agreement with previous studies in Tanta University Hospitals. For instance, Emara *et al.*[17] also reported higher prevalence of Gram-negative isolates in CA and HA infections. In the current study, *S.aureus* was the most commonly isolated Gram-positive organism (60.96%), being 68.49% and 53.42% of CA and HA Gram-positive isolates, respectively. This result came consistent with many other studies, including Abd-Elmonsef *et al.*[18] (92.8%), Ali *et al.*[19] (65.27%), and Xie *et al.*[20] (87.1%).

In the present study, extremely high prevalence of MDR was observed in Gram positive isolates of both CA and HA infections, being 65.75% and 72.6%, respectively. These percentages are more or less similar to those detected by Azzab *et al.*[21] who detected a percentage of MDR among their Gram-positive isolates from Zagazig University Hospitals to be 65.2%.

Linezolid-resistant isolates were detected at a percentage of 1.37% among all Gram-positive isolates in the current study. All CA Gram-positive isolates were susceptible to linezolid. On the other hand, two (2.74%) HA Gram-positive isolates were linezolid-resistant. One of them was *S.aureus* isolate and the other was CoNS; thus, the rate of linezolid resistance among *Staphylococci* isolated in this study was 1.69%. All *Enterococci* were linezolid-susceptible.

In agreement with the current study, Maarouf *et al.*[22] reported a percentage of 1.29% linezolid resistance among the Gram-positive isolates from patients of Alexandria Main Hospitals. Conversely, Azzab *et al.*[21]

detected 100% linezolid susceptibility among all Gram-positive isolates in Zagazig University. However, this could be explained by the fact that their sample size was small (65 patients), with just 23 Gram-positive isolates. Another reason is that their study was carried out five years earlier to the current study.

In the current study, *cfr* gene was detected in the two linezolid-resistant isolates. Similarly, Mittal *et al.*[23] detected *cfr* gene in 100% of their linezolid-resistant CoNS isolates. The epidemiological significance of this finding is that *cfr* carrying *Staphylococci* caused many nosocomial outbreaks, such as a large outbreak that occurred in an ICU in Madrid due to clonal expansion of *cfr*-carrying MRSA, beside horizontal transmission of *cfr* to other clones of MRSA[24].

In this study, the two linezolid-resistant isolates were both *optrA*-negative and *poxtA-negative*. These results were in partial agreement with Ding *et al.*[25] who detected 21 linezolid-resistant staphylococcal isolated; 12 of them were positive for *cfr*, while all were *optrA* and *poxtA* negative.

Interestingly, the linezolid-resistant CoNS detected in the current study was isolated from a patient who was prescribed linezolid as an empirical therapy for upper respiratory tract infection. In the same context, Zahran *et al.*[6] reported the linezolid prescribing practices to be extremely abusive in a hospital in Saudi Arabia where linezolid was the antibiotic to be most often prescribed for cases of catheter-associated urinary tract infections. Similarly, Kramer *et al.*[7] analyzed the prescribing practices of linezolid in German hospitals and concluded that during the period from 2011 to 2016, linezolid prescribing has significantly increased.

This study is limited by the fact that linezolid-resistant isolates were not assessed for the presence of chromosomal mutations in 23s rRNA that may play a role in their resistance to linezolid. Thus, we recommend that future studies assessing genotypic resistance to linezolid take this point into consideration.

CONCLUSION

Linezolid resistance was only detected in two Gram-positive pathogens isolated from cases with nosocomial infections included in this study. Both of them were *Staphylococci*, positive for *cfr* gene and negative for *optrA* and *poxtA* genes. Thus, linezolid, like all other antibiotics, is not immune against development of resistance. If linezolid abuse and extensive use in clinical practices continue in the same way, linezolid efficacy is expected to decrease with time to an extent that can threaten its being as one of the antibiotics of last resort.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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