ORIGINAL ARTICLE

Antibiotic Susceptibility Pattern and Biofilm Production of Multidrug- Resistant Organisms (MDROs) Isolated from Suez-Canal University Hospitals

[1]Asmaa A Hashem*, [1, 2]Samaa A Taha, [3]Maha M Anani

[1]Department of Microbiology and Medical Immunology, Faculty of Medicine, Suez- Canal University, Ismailia 41522, Egypt
[2]Department of Microbiology, College of Medicine, Taif University, Al-Taif, Saudi Arabia
[3]Department of Clinical Pathology, Faculty of Medicine, Suez- Canal University, Ismailia 41522, Egypt

Key words:
Biofilm, Bacterial susceptibility, MDROs

Corresponding Author:
Asmaa A. Hashem
Telephone: +201064027524
ORCID of asmaa hashem: 0000-0002-0547-2096
asmamicro82@gmail.com
asmamicro@yahoo.com

ABSTRACT

Background: *The increase of morbidity of infected patients with multi-drug resistant organisms (MDROs) necessitated use of alternative drugs as tigacycline and colistin.* ***Methodology:*** *A total of 172 isolates were tested for detection of MDR, XDR, and PDR bacteria, testing biofilm-formation by CRA and TCP methods, combined disc test for ESBL production among Gram negative; Cefoxitin disk diffusion and PCR for presence of the mecA gene among Gram positive strains. Disc diffusion method was done to assess susceptibility to Tigecycline and Colistin.* ***Results:*** *Variable drug resistance 45% XDR and 23% MDR for Gram negative and 28%, 19%, respectively for Staphylococcus species was recorded. The high sensitivity to Tigacycline was found among all Klebsiella, E. Coli and Enterobacter isolates, also to all isolated Staphylococcal species, while highest colistin sensitivity was noticed in 33% of ESBL producing Enterobacter.* ***Conclusion:*** *Increased prevalence of MDROs. Tigacycline was highly active against MDROs rather than colistin.*

INTRODUCTION

Antibiotic resistance is a serious problem, the misuse or overuse of antibiotics leads to the development of resistant or super-resistant bacterial strains[1].

A rise of the prevalence of multidrug‑resistant organisms (MDROs) infections and its consequences like consumption of healthcare resources through prolonged hospital stay [2].

The European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC), describe acquired resistance profiles in multidrug-resistant organisms [3].

Multidrug- resistant organisms MDROs have been divided into three categories: Multi drug resistance (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, extensive drug-resistance (XDR) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories and pan-drug resistance (PDR) was defined as non-susceptibility to all agents in all antimicrobial categories [3].

Polysaccharide Intercellular Adhesin (PIA) is an exo-polysaccharide matrix of microbial and host origin where microorganisms are accumulated forming the biofilm [4], Gram positive cocci, Gram negative bacilli and *Candida albicans* are all bacterial pathogens that are capable of forming biofilm [5].

A biofilm make organisms resistant to anti-microbial agents through failure of antibiotics to penetrate the polysaccharide matrix and also growth of cells of the biofilm in starved state, exchange of plasmids responsible for drug resistance due to adherence of cells increases the anti-microbial resistance[6], also induction of a biofilm phenotype characterized by activated multidrug-efflux pumps and altered membrane-protein composition [7].

Tigecycline, a derivative of minocycline, is one of the glycylcycline classes of antibiotics, acts as an inhibitor of bacterial protein synthesis, it is bound to the same high-affinity site as tetracycline in the16S rRNA [8].

In vitro and in vivo studies have shown that Tigacycline has broad spectrum of activity against a variety of Gram-positive and Gram-negative aerobic MDROs [9], anaerobic and atypical pathogens, including vancomycin-resistant enterococci, methicillin-resistant Staphylococcus aureus, penicillin- resistant *Streptococcus pneumoniae*, Enterobacteriaceae, non-Enterobacteriaceae except Pseudomonas[9].

Consequent to an increasing number of infections with multi-resistant Gram-negative bacteria [10], Colistin was used as an alternative antibiotic for treatment of MDROs [11].

Colistin (polymyxin E), was proven to be effective for treatment of infections caused by *Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella penumoniae, Escherichia coli, Enterobacter sp,*

Salmonella sp, Shigella sp, and Haemophilus influenzae.

This study aims to detect the antibiotic susceptibility pattern, different resistance profiles, mechanisms of resistance , biofilm Production of Multidrug- Resistant Gram negative and Gram positive organisms causing hospital acquired infections and to determine role of Tigacycline and Colistin as alternative line of treatment to MDROs.

METHODOLOGY

Bacterial isolates:

The study was conducted in Suez- Canal University Hospitals from September 2016 to May 2018.A total of 172 non-repetitive, clinically significant isolates from hospitalized patients were included in the study.

The organisms were identified by conventional methods. The organisms which were tested included Gram negative bacteria included *E.coli* (n= 62), *Klebsiella spp* (n=28), *Pseudomonas* (n=20) and *Enterobacter* (n=4). The source of these isolates included urine (n=65), sputum (n=21), pus (n=17) and blood (n=11); *Staphylococcus* isolates which recovered from Catheter Related Blood Stream Infections (CRBSI); *S. aureus* (n=20), *coagulase negative staphylococci* (CoNS) (n=38) (*S. epidermidis, S. hemolyticus, S. schleiferi, S. warnei and S. Lugdunensis).*

Anti-microbial susceptibility testing:

Antibiotic susceptibility testing was done using standard Kirby-Bauer disk diffusion method and interpreted according to the CLSI guidelines[12], Antibiotic discs (Oxoid, Basingstoke, UK)for Gram positive strains included; clindamycin (DA 2µg), erythromycin (E 15µg), rifampin (RF 5µg),gentamycin (CN 10µg), ciprofloxacin (CIP 5µg), tetracycline (TE 30µg) and cotrimoxazole (SXT 25µg).

Gram negative bacterial strains were tested for the following 5 classes of antibiotics: Cephalosporins (ceftazidime (CAZ 30µg), cefotaxime (CTX 30µg) and cefepime (FEP 30µg)); Monobactams (aztreonam (ATM 30 µg),piperacillin (PRL100µg); Carbapenms: imipenem (IMP10µg) and meropenem (MEM 10µg); Aminoglycosides (gentamicin (CN 10 µg), tobramycin (TOB 10µg) and amikacin (AK 30µg) andFluroquinolones (nalidixic acid (NA 30µg), norfloxacin (NOR 10µg), levofloxacin (LEV 5µg), ciprofloxacin (CIP 5 µg)and ofloxacin (OFX 5 µg).

Test for biofilm production

All isolates were screened for their ability to form biofilm by the Congo Red Agar (CRA) method[13] and the Tissue Culture Plate (TCP)[14] methods respectively.

Extended-spectrum beta-lactamase (ESBL) detection

All the Gram negative strains were screened for ESBL production using cefotaxime (CTX 30µg) and ceftazidime (CAZ 30 µg). Strains showing a zone of inhibition of ≤ 27 mm for CTX and ≤ 22 mm for CAZ were selected for ESBL combined disc conformation test. Combined discs of CTC (40 µg) and CZC (40 µg) were used in the confirmation test according to the CLSI M2-A10 guideline [15]. Isolates of *K. pneumoniae* were tested for ESBL production in a previous work by the standard disc diffusion method in addition to the ESBL NDP test and flow cytometric assay [16]. K. pneumoniae ATCC 700603 and *E. coli* ATCC 25922 were used as ESBL- positive and negative, respectively.

Methicillin resistance:

It was detected in *Staphylococcus* and CoNS strains using disc diffusion method cefoxitin (FOX 30µg) (Oxoid, Cambridge, UK), the results were interpreted in accordance with CLSI guidelines[17]. Theses strains were analyzed by PCR for the presence of the mecA gene in a previous study[18].

Susceptibility to Tigecycline and Colistin

Disc diffusion susceptibility testing was performed for all the isolates using Tigecycline (TGC) disks (15 µg; Oxoid Ltd, Basingstoke, Hants, UK). The interpretation of zone diameters for all Gram negative bacteria was done using the US FDA Tigecycline susceptible breakpoints listed for Enterobacteriaceae (≥ 19 mm zone size). Resistance was defined as zone size ≤ 14 mm. Interpretation of zone diameters of all Gram-positive bacteria were done using the US FDA Tigecycline susceptible breakpoints listed for *S. aureus* (≥ 19 mm zone size) according to [19]. For colistin susceptibility, disk diffusion testingwas performed for all Gram negative bacteria using colistin disks (CST 10µg)according to the EUCAST guidelines[20].

Statistical analysis:

Data were organized in a database file, and analyzed using Statistical Package for Social Science (SPSS) for Windows (version 16.0; Chicago, SPSS Inc) software program. As for qualitative data, distribution was calculated. The chi square test was used and the level of significance was considered at p-value <0.05% to compare between groups.

RESULTS

This study was carried out on 172 non repetitive isolates. Gram negative organisms comprised 114 (66%) isolates; while Gram positive organisms comprised 58 (34%) isolates. Staphylococcus isolates recovered from Catheter Related Blood Stream Infections (CRBSI), were twenty (34.5%) *S. aureus* and 38 (65.5%) strains of *coagulase negative staphylococci* (CoNS): (20) *S. epidermidis*, (9) *S. hemolyticus* strains, (3) *S. schleiferi* strains, (2) *S. warnei strains*,and (4) strains of *S. Lugdunensis*.

Isolated Gram negative organisms:

Sixty two (54%) *E.coli* strains, 28 (25%) *Klebsiella spp*., 20 (17%) *Pseudomonas*, finally 4 (4%) *Enterobacter* strains (Table 1). *Klebsiella pneumoniae*

was the most frequent isolated *Klebsiella* species (n=21) (75%), followed by *K.oxytoca* (n=6) (22%) and only one isolate was *K.ozaene* (3%) (Table 2).

Table 1: Frequency and percentage of Gram negative strains

	Frequency	Percentage
E.coli	62	54%
Klebsella. spp.	28	25%
Pseudomonas	20	17%
Enterobacter	4	4%
Total	114	100%

Table 2: Frequency and percentage of Klebsella. spp.

Klebsella. spp.	**Frequency**	**Percentage**
K.pneumonia	21	75
K.oxytoka	6	22%
K.ozaene	1	3%
Total	28	100%

Biofilm production among Gram negative bacteria were 59% by CRA (59 out of 114) and 63% by TCP (72 out of 114) respectively. Biofilm production was prominent among Klebsiella spp. 75% and 72% by CRA and TCP, respectively. *Pseudomonas* strains showed biofilm production by CRA and 45% and 65% by TCP, respectively (Table 3).

ESBL production was founded among 62.3% (71 out of 114) of isolated Gram negative bacteria, The commonest species were *Enterobacter* (75%), *E. coli* (68%) and *Pseudomonas* (60%)

Drug resistance among isolated Gram negative bacteria were 45% XDR, 23% MDR and 7% PDR.Especially MDR and XDR were predominantly noticed among *Pseudomonas* (50%, 40%) and *E.coli* (27%, 48%) while PDR was noticed among *Enterobacter* (25%) and *Klebsiella.spp* (18%) (Table 3).

Table 3: Percentage of biofilm production, ESBL, MDR, XDR, PDR among Gram negative isolates

	Biofilm production		**ESBL**	**MDR**	**XDR**	**PDR**
	CRA	**TCP**	**62%** **71/114**	**23%** **26/114**	**45%** **51/114**	**7%** **8/114**
E.coli N= (62)	45%	58%	68%	27%	48%	2%
Klebsiella N= (28)	75%	72%	50%	25%	32%	18%
Pseudomonas N= (20)	45%	65%	60%	50%	40%	5%
Enterobacter N= (4)	25%	75%	75%	25%	0%	25%

E.coli *strains showed antibiotic resistance to cephalosporins, carbapenems, Monobactam, Piperacillin higher among ESBL Biofilm producing* E.coli *strains than other groups; higher resistance to fluroquinlones, monobactam among ESBL producers with statistically significant difference to Cephalosporins, Tobramycin, Pipracillin and Imipinem (Table 4).*

Klebsiella isolates showed higher antibiotic resistance among ESBL Biofilm producers, except resistance to carbapenemes and fluroquinilone were higher among non ESBL non Biofilm producers with statistically significant difference to Meropenem and Imipinem.

Pseudomonas isolates showed higher antibiotic resistance among ESBL Biofilm producers rather than other strains, with statistically significant difference to Pipracillin-Aztreonam Cefepime,Nalidixic acid and Fluroquninilones.

Enterobacter isolates showed antibiotic resistance among non ESBL non biofilm producers, with statistically significant difference to Aminoglycosides, Amikacin, Aztreonam and Fluroquninilones (Table 4).

Table 4: Antibiotic resistance pattern among ESBL biofilm, ESBLonly, biofilm only and non- ESBL non- biofilm producers Gram negative isolates

	ESBL BIOFILM				ESBL ONLY				BIOFILM ONLY				NON ESBL NONBIOFILM			
	E. coli	Klep	Pseudo	Entero	E. coli	Klep	Pseudo	Entero	E. coli	Klep	Pseudo	Entero	E. coli	Klep	Pseudo	Entero
# of Resistant strains	38	14	12	3	4	0	0	0	2	5	1	0	18	9	7	1
CTX	**87% *****	86%	100%	100%	75%	0%	0%	0%	0%	60%	100%	0%	56%	77%	100%	100%
CAZ	**95% *****	93%	100%	100%	75%	0%	0%	0%	0%	60%	100%	0%	56%	78%	86%	100%
FEP	**84% *****	64%	**92% *****	67%	75%	0%	0%	0%	0%	80%	0%	0%	50%	89%	0%	100%
CN	**34%**	43%	**100% *****	0%	25%	0%	0%	0%	0%	40%	0%	0%	44%	44%	14%	**100% *****
TOB	**45% *****	57%	58%	0%	75%	0%	0%	0%	100 %	80%	0%	0%	83%	44%	14%	**100% *****
AK	3%	29%	75%	0%	0%	0%	0%	0%	0%	40%	100%	0%	6%	22%	42%	**100% *****
MEM	45%	29%	25%	67%	0%	0%	0%	0%	0%	60%	0%	0%	22%	**78% *****	14%	100%
IMP	**0% *****	7.%	50%	33%	0%	0%	0%	0%	0%	60%	0%	0%	22%	**44% *****	14%	100%
PRL	**82% *****	79%	**75% *****	33%	75%	0%	0%	0%	50%	100%	0%	0%	72%	89%	14%	100%
ATM	74%	57%	**83% *****	0%	75%	0%	0%	0%	0%	80%	0%	0%	56%	78%	0%	**100% *****
NA	71%	29%	**100% *****	33%	75%	0%	0%	0%	0%	60%	0%	0%	67%	56%	0%	**100%**
NOR	66%	21%	**100% *****	0%	75%	0%	0%	0%	0%	60%	0%	0%	61%	44%	0%	**100% *****
CIP	66%	21%	**100% *****	0%	75%	0%	0%	0%	0%	60%	0%	0%	61%	44%	0%	**100% *****
LEV	66%	21%	**100% *****	0%	75%	0%	0%	0%	0%	60%	0%	0%	61%	44%	0%	**100% *****
OFX	66%	21%	**100% *****	0%	75%	0%	0%	0%	0%	60%	0%	0%	61%	44%	0%	**100% *****

*** Statistical significance difference (P value < 0.05)

Percentage of MDR, XDR (82%, 67%) among ESBL biofilm producers E.coli isolates were higher than non ESBL biofilm producers with statistically significant difference (p value =0.048).

Percentage of MDR, XDR (71%, 56%) among ESBL biofilm producers klebsiella spp. isolates were higher than non ESBL biofilm producers without a statistically significant difference (P value =0.330).

Among Pseudomonas isolates percentage of MDR (60%) were higher among non ESBL biofilm producers; whileXDR was (100%) among ESBL biofilm producers Pseudomonas isolates, with statistically significant difference(P value =0.070).

All MDR Enterobcter isolates were ESBL biofilm producers, while XDR isolates were non ESBL biofilm producers without a statistically significance difference (P value =0.135). The results are shown in table 5.

Table 5: Relation between drug resistance (MDR,XDR) and ESBL Biofilm producers and non-producers

Antibiotic Resistance	ESBL BIOFILM				Non ESBL, Non BIOFILM			
	E. coli	***Klep***	***Pseudo***	***Entero***	***E. coli***	***Klep***	***Pseudo***	***Entero***
MDR	82%	71%	30 %	100%	12%	14%	60%	0%
XDR	67%	56%	100%	0%	27%	33%	0%	100%
P- value	0.048	0.330	0.070	0.135	0.048	0.330	0.070	0.135

The high sensitivity to Tigacyclin (TGC) was found among all Klebsiella isolates (ESBL, MDR, XDR), E.coli isolates (ESBL, MDR) and Enterobacter isolates **(Figure 1)**. Colistin sensitivity is noticed among 33% of ESBL producing Enterobacter, and (8% ESBL, 10% MDR, 12.5% XDR) Pseudomonas isolates **(Figure 2)**.

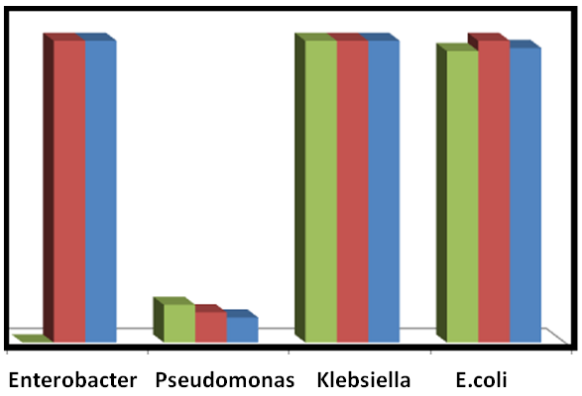

Fig. 1: TGC sensitivity in (ESBL, MDR, XDR) among Gram negative isolates.

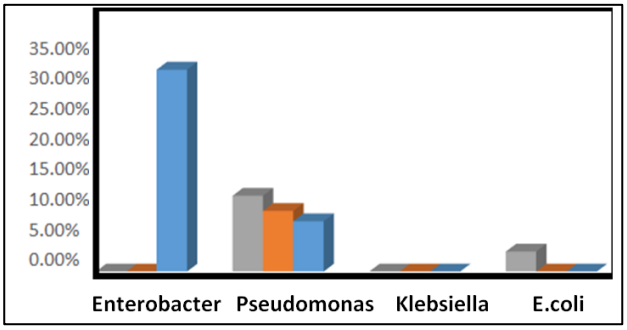

Fig. 2: Colistin sensitivity among (ESBL, MDR, XDR) Gram negative isolates

Among (58) *Staphylococcus* strains 33 (57%) and 37 (64%) were biofilm producers by CRA and TCP methods respectively; furthermore Percentage of MDR and XDR were19% (11/58), 28% (16/58) respectively; moreover the Cefoxitin resistance was detected among 25 (43%) Staphylococcus strains while the mecA gene was found in 19 (33%) isolates only.

All isolated staphylococcal species, including biofilm producers and methicillin resistant isolates were susceptible to Tigacyclin.

Antibiotic resistance pattern according to Methicillin resistance and biofilmproduction

Isolated *Staphylococcus* spp. were classified into Four groups according to biofilm production and methicillin resistance (Table 6).

Table 6: Biofilm forming abilities and methicillin resistance (mecA gene) for *Staphylococcus* isolates

	Frequency	**Percentage**
Biofilm Only	22	38%
Biofilm Producer and Methicillin Resistant	15	26%
Methicillin Resistance Only	4	7%
Non Methicillin Resistant- non Biofilm Producer	17	29%
Total	58	100%

The highest antibiotic resistance was noticed among biofilm producing staphylococcus isolates rather than other groups, with statistically significant difference to clindamycin. Among twenty two biofilm producers 73% were resistant to erythromycin, while 59% were resistant to clindamycin, gentamycin and tetracycline. Fifteen (47%) methicillin resistant biofilm producing isolates were resistant to gentamycin and tetracycline, while 40% were resistant to erythromycin (Table 7).

Table 7: Antibiotic resistance pattern according to Methicillin resistance (MR) and biofilm production

Antibiotic Resistance	MR Biofilm 15		MR Only 4		Biofilm Only 22		Non MR- Non Biofilm 17		P-VALUE SIG<0.05
	NO	%*	NO	%*	NO	%*	NO	%*	
Clindamycin	3	20%	1	25%	13	59%	4	24%	0.044*
Erythromycin	6	40%	3	75%	16	73%	13	77%	0.117
Rifampin	3	20%	1	25%	5	23%	5	29%	0.935
Gentamycin	7	47%	2	50%	13	59%	10	59%	0.871
Ciprofloxacin	5	33%	1	25%	10	46%	5	29%	0.700
Tetracycline	7	47%	1	25%	13	59%	6	35%	0.391
Cotrimoxazole	4	27%	1	25%	10	46%	6	35%	0.653

DISCUSSION

The major challenges in the therapy and control of nosocomial infections are antimicrobial resistance and the emergence of multi-drug resistant organisms.

In this study, *E. coli, Klebsiella.spp and pseudomonas* were the most common Gram negative organisms causing different nosocomial infections as follows, UTI (57%) followed by pneumonia (18.4%), SSI (17%) and septicemia (9.6%), while Coagulase negative *Staphylococcus and S. aureus* were the commonest causing Catheter Related Blood Stream Infections (CRBSI).Our findings are consistent with Balkhair [21].

Biofilm producing Gram negative bacteria in this study were 51.8%, 63.2% by CRA and TCP, respectively. Using the TCP method as a gold standard method, biofilm production was higher among Klebsiella spp. (72%), Pseudomonas (65%) and *E.coli* strains (58%).These findings are consistent with Venkata [22], also Carlos et al. [23] found the highest biofilm producers was *Pseudomonas* spp (83%),

In this study, ESBL was found among 71 /114 (62%) of isolated Gram negative bacteria, mainly among *Enterobacter (75%), E.coli (68%), pseudomonas (60%) and Klebsiella spp (50%).*

These results are similar to a study conducted by Nesma et al.[24] who found that the rate of ESBL was 48%, also the highest species were *E.coli* 50%, *Klebsiella* 48% and 33% *Enterobacter* 33% .

The biofilm prevents antimicrobial agents from entering the bacterium, also protect bacteria from the host's immune system, leading to persistent infections [25]. All our ESBL biofilm Gram negative producers showed more resistance to the most tested antibiotics, Likewise ESBL producers *E.coli* strains showed higher resistance to fluroquinlones and monobactam.

Sundaram et al. [26] found that most of the biofilm producers were multiple drug resistant; Sabina Fatima[27] found that drug resistance and biofilm production are directly proportional and 54% of MDR isolates were found to be biofilm producers .

Our percentage of MDR, ESBL among Gram negative bacteria were (23%, 62%) respectively. Giuffrè et al.[28] found in their study the prevalences of MDR, ESBL producing GNB were 28.8% and 11.7% respectively. In our study XDR is 45%, it is mainly noticed among *E. coli, Pseudomonas and Klebsiella* spp. respectively.

Our findings are consistent with a study conducted by Sabina Fatima et al.[27],who reported that 32.6% of *E. coli* and 25% of Klebsiella spp were MDR, followed by Pseudomonas spp; Jaggi et al. [29] in their study found that Kelbsiella spp and E.coli were highest multi-drug resistance organisms as a result of ESBL production. Basak et al.[30] found 33.5%, 12.1% of GNB bacteria were MDR, XDR respectively , no PDR strain was detected, also the commonest MDR species were *E. coli* (31.6%), and *Klebsiella pneumoniae* (30%) .

Additionally, MDR among ESBL biofilm producers were higher than the non producers in *Enterobacter, E. coli and Klebsiella spp.* Also XDR among ESBL biofilm producers were highly noticed in Pseudomonas, E.coli and klebsiella spp.

Balkhair [21] reported that ESBL producers were highly resistance to quinolones and piperacillin/ tazobactam, considering them as multidrug-resistance organism.

Considering methicillin resistant and biofilm producers *Staphylococcus* as members of MDRO 33% and 64% of our strains carry mecA gene and biofilm producers respectively. In the light of drug resistance 19% and 28% were MDR and XDR, respectively.

The Biofilm producing group showed higher resistance rather than other groups to tested clindamycin, gentamycin, tetracycline (59%); as well as ciprofloxacine and cotrimoxazole (45.5%) respectively. These results are similar to Oliveira[31] who found that biofilm produces Staphylococcal species have high and variable multi-drug resistance, mainly to oxacillin (69.4%), erythromycin (40.8%) ,gentamycin (36.7%), sulfamethoxazole/trimethoprim (16.3%).

Jinnethe et al. [32] found that the prevalence of erythromycin and clindamycin resistance in *S. aureus*

was (58%, 57%), and in Coagulase-negative Staphylococci were (63.4%, 45.1%) respectively. moreover all isolated *Staphylococcal* species, including biofilm producers and methicillin resistant isolates were susceptible to Tigacycline. Tigacycline was active against all MRSA isolates from complicated SSTIs [33]. As well as, Livermore[34] noticed that itis a potent antimicrobial agents, when it is compared with linezolid and vancomycin, it exhibits greater activity than linezolid against vancomycin-resistant *E. faecalis and E. faecium (VRE),* also it can be used as empiric treatment of serious infections sustained by some of the commonly encountered pathogens.

Equally important, highest Tigacycline sensitivity was detected among all Klebsiella (ESBL, MDR, XDR) ,E.coli (ESBL, MDR) and Enterobacter isolates; Tigacycline has a bacteristatic action with wide antibacterial activity not only against Gram-positive organisms, but also against Gram-negatives [35].

Comparatively, in our result, Colistin sensitivity was noticed among 33% of ESBL producing *Enterobacter*, and (8%, 10%, 12.5%) ESBL, MDR, XDR *Pseudomonas* isolates, all our Klebsiella sp.,and 97% of E.coli were colistin resistant. Linden [36] reported that the increasing systemic use of colistin against MDR pathogens, lead to development of resistance in nosocomial strains .

Nachimuthu et al.[37] reported that resistance to colistin was 29%, and it included different species *E. coli, K. pneumoniae, P. aeruginosa, A. baumannii, P. mirabilis*, . In the same way colistin resistance in K.pneumonia ranged from 6.8% in South Korea [38], to 27% in Australia [39].

In conclusion, this study shows an increase of multidrug‑ resistant organisms (MDROs). A strong relation was detected between ESBL producers,biofilm producers,MRSAand MDROs.Tigacycline was highly active against MSSA , MRSA isolates, *Klebsiella* , *E.coli and Enterobacter* isolates.Moreover Colistin sensitivity is noticed in ESBL producers *Enterobacter*, XDR *Pseudomonas* isolates,while all *Klebsiella* sp.,and most of *E.coli* were colistin resistant.This increasing resistance patterns highlight the importance of antibiotic policy and guidelines, also aggressive adherence to infection control strategies and practices.

REFERENCES

1. Habibzadeh, F. Lancet (London, England). http://www.the lancet. com/pb/assets/raw/Lancet/global‑health/middle‑ east/Nov13_MiddleEast/:2013 Ed.pdf elsca1=220713
2. Gabor, Z., Antonio, A.and Ignacio, M. L. What's new in multidrug-resistant pathogens in the ICU? *Ann. Intensive Care 2016;*6:96 DOI 10.1186/s13613-016-0199-4.
3. Magiorakos, A‑P., Srinivasan, A., Carey, R.B.,et al. Multidrug‑resistant, extensively drug‑resistant and pandrug‑ resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012;18:268–81.
4. Samant, S. A. and Pai Chitra G. Evaluation of different detection methods of biofilm formation in clinical isolates of *Staphylococci .Int J Pharm Bio Sci* Oct 2012 ; 3(4): (b) 724 – 733.
5. Bailey and Scott's. Diagnostic Microbiology, 13th edition by Patricia M. Tille. Elsevier publications 2014; 35.
6. Venkata, H.N., Parvathi T. and Perala B.K. Study of Biofilm Production and Anti-microbial susceptibility pattern of bacterial and fungal isolates from urinary catheters. *Int.J.Curr.Microbiol.App. Sci.2016;* 5(2): 415-424. doi: http://dx.doi.org/10.20546/ijcmas.2016.502.046.
7. Stewart, P.S., (2002). Mechanisms of antibiotic resistance in bacterial biofilms. *Int J Med Microbiol 2002;* 292(2):107–13.
8. Bauer, G., Berens,C., Projan, S. J., and W. Hillen. Comparison of tetracycline and tigecycline binding to ribosomes mapped by dimethylsul- phate and drug-directed Fe2 cleavage of 16S rRNA. *J. Antimicrob. Chemother2004;* 53:592–599.
9. Bradford, P. A. Tigecycline: a first in class glycylcycline. *Clin. Micro- biol. Newsl 2004;*26:163–168.
10. Li, J., Nation, R.L., Turnidge, J.D., et al. Colistin: the re-emerging antibiotic for multidrug resistant Gram negative bacterial infection. Lancet. 2006 9(6):589- 601.
11. Falagas, E.M., Kasiakou, S.K. Colistin: the revival of polymyxins for the management of multidrug resistant Gram-negative bacterial infections. Clin Infect Dis 2005;40(5):1333-41.
12. CLSI-Clinical and Laboratory Standards Institute. M100-S24 Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement.CLSI 2014; Wayne, PA.
13. Hassan, A., Usman, J., Kaleem, F., Omair, M., Khalid, A., Iqbal, M.Evaluation of different detection methods of biofilm formation in the clinical isolates.*Braz J Infect Dis 2011;*15 (4): 305-11.
14. Merritt, J.H., Kadouri, D.E., O'Toole, G.A. Growing and analyzing static biofilms. *Curr ProtocMicrobiol 2005;* B.1.1-1B.1.17.

15. CLSI-Clinical and Laboratory Standards Institute . Performance standard for antimicrobial disk susceptibility tests. CLSI 2009; M2-A10. Wayne.
16. Taha, S., Youssef, N., Elkazaz, A., Ramadan, H. Detection of extended-spectrum beta-lactamases (ESBLs) in clinical isolates of Klebsiellapneumoniae using the ESBL NDP test and flow cytometric assay in comparison to the standard disc diffusion. Afr J Microbiol Res 2015; 9(34):1947–53. doi: 10.5897/AJMR2015.7691.
17. CLSI. Performance Standards for Antimicrobial Susceptibility Testing, (M100-S23), vol. 33, No. 1. Wayne, PA: CLSI 2013; Wayne, PA.
18. Hashem,A.A.,Abd El Fadeal,N.M., Shehat ,A.S. In vitro activities of vancomycin and linezolid against biofilm producing methicillin-resistant staphylococci species isolated from catheter-related bloodstream infections from an Egyptian tertiary hospital. *Journal of Medical Microbiology 2017;* 66:744–752.DOI 10.1099/jmm.0.000490.
19. Wyeth Pharmaceutics . Tygacil (Tigecycline) for Injection [package insert] Philadelphia, PA: Wyeth Pharmaceuticals Inc; 2005.
20. EUCAST. 1 January . Breakpoint tables for interpretation of MICs and zone diameters, version 5.0. EUCAST Laboratory for Antimicrobial Susceptibility Testing, Växjö, Sweden 2015 .
21. Balkhair A., Al-Farsi Y.M., Al-Muharrmi Z.(2014) .Epidemiology of Multi-Drug Resistant Organisms in a Teaching Hospital in Oman: A One-Year Hospital-Based Study. *Scientific World Journal 2014;*Volume 2014, 6 pages, Article ID 157102, http://dx.doi.org/10.1155/2014/157102
22. Venkata, H.N., Parvathi, T., and Perala, B., K. Study of Biofilm Production and Anti-microbial susceptibility pattern of bacterial and fungal isolates from urinary catheters. *Int.J.Curr.Microbiol.App.Sci 2016;* 5(2): 415-424.doi: http://dx.doi.org/10.20546/ijcmas.2016.502.046
23. Carlos, J. S., Katrin, M., Miriam, L. B., et al. Biofilm formation by clinical isolates and the implications in chronic infections.*BMC Infectious Diseases2013; 13:47, 1-12.*
24. Nesma, S., kiran,T., and Dhanvijay, A.G. Study of ESBL producing Enterobactericea and antibiotic coresistance in tertiary care teaching hospital. *J Nat Sci Bio Med* 2014;5(1):30-35.
25. Antunes, A.L., Trentin, D.S., Bonfanti, J.W., Pinto, C.C., Perez, L.R., Macedo, A.J.; Barth, A.L. Application of a feasible method for determination of biofilm antimicrobial susceptibility in staphylococci. APMIS 2010 , 118,873–877.
26. Sundaram, M., Babu, D., Dhandapani, K.A. study on correlation between the drug resistance and biofilm production among the GNB isolated from blood. *Indian J Microbiol Res* 2016 ;3(2):197-202.
27. Sabina Fatima, K. P., and Nagamani.K.Comparative evaluation of biofilm production in Multidrug resistant and sensitive Gram negative clinical isolates .*Int.J.Curr. Microbiol.App.Sci 2015;* 4(6): 918-926
28. Giuffrè, M., Geraci, D. M., Bonura, C., et al. The Increasing Challenge of Multidrug-Resistant Gram-Negative Bacilli: Results of a 5-Year Active Surveillance Program in a Neonatal Intensive Care Unit. *Medicine 2016;95* (10), e3016. http://doi.org/10.1097/MD.0000000000003016
29. Jaggi,N., Pushpa,S., Lalit, S. Control of multidrug resistant bacteria in a tertiary care hospital in India. *Journal of Antimicrobial Resistance and Infection Control 2012,* 1:23, 2-7.
30. Basak, S., Singh, P., & Rajurkar, M. Multidrug Resistant and Extensively Drug Resistant Bacteria: A Study. *Journal of Pathogens* 2016, Article ID 4065603, 5 pages. http://doi.org/10.1155/2016/4065603
31. Oliveira, A., Pereira, V.C., Pinheiro, L., et al . Antimicrobial Resistance Profile of Planktonic and Biofilm Cells of *Staphylococcus aureus* and Coagulase-Negative *Staphylococci*. *Int J Mol Sci*. 2016; Sep; 17 (9): 1423.
32. Jinnethe, R., Marylin, H., Lorena, Díaza et al . Characterization of macrolide resistance in Gram-positive cocci from Colombian hospitals: a countrywide surveillance. *International Journal of Infectious Diseases* 2007;11, 329—336
33. Renteria, M., Biedenbach, D.J., Bouchillon, S.K., *et al.*. In vitro activity of tigecycline against isolates collected from complicated skin and skin structure infections and intra-abdominal infections in Africa and Middle East countries: TEST 2007-2012. *Diagn Microbiol Infect Dis 2014;* 79,54-59.
34. Livermore, D., M. Tigecycline: what is it, and where should it be used? *J Antimicrob Chemother 2005;* 56 ,: 611- 14.
35. Wyeth Pharmaceuticals . Tygacil 50mg powder for solution for infusion 2007; Retrieved from http://emc.medicines.org.uk
36. Linden, P.K., Kusne, S., Coley, K., et al .(2003). Use of parenteral colistin for the treatment of serious infection due to antimicrobial-resistant *Pseudomonas aeruginosa*. *Clin Infect Dis 2003;*37:e154.
37. Nachimuthu, R., Manohar, P., Subramani, R., et al.. Colistin susceptibility of gram-negative clinical

isolates from Tamil Nadu, India. *Asian Biomedicine 2016;*Vol. 10 , 35 - 39

38. Poudyal, A., Howden, B., Bell, J., et al. *In vitro* pharmacodynamics of colistin against multi-drug resistant *Klebsiella pneumoniae*. *J Antimicrob Chemother 2008;* 62:1311-8.

39. Suh, J-Y., Son, J.S., Chung, D.R., et al . Nonclonal emergence of colistin resistant *Klebsiella pneumoniae* isolates from blood samples in South Korea. *Antimicrob Agents Chemother2010;* 54:560-2.