First report of VIM metallo-β-lactamase production in *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates from Gaza Strip, Palestine

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Abstract

Introduction **Even though the increasing incidence of VIM-producing *E. coli* and *K. pneumoniae* has been reported worldwide, studies are still lacking in Palestine. The aim of this study was to screen carbapenem-resistant *E. coli* and *K. pneumoniae* bacteria in the Gaza Strip, Palestine and further to characterize carbapenemase-producing isolates.**

Methods **A total of 69 *E. coli* and 27 *K. pneumoniae* isolates were obtained from three Gaza hospitals and recovered from urine, wound swabs, blood and ear discharge. The screening for metallo-β-lactamases (MBLs) was performed by using the imipenem-EDTA disc synergy test. The detection of β-lactamases genes, detection of non-β-lactam genes and the characterization of integrons were performed by PCR and sequencing. The clonal relationship among the isolates was determined by pulsed-field gel electrophoresis (PFGE).**

Results **Our study showed that 4 *E. coli* (5.8%) and 5 *K. pneumoniae* (18.5%) were positive by the imipenem-EDTA disc synergy test. *Bla*$_{VIM-4}$ was detected in six isolates and *bla*$_{VIM-28}$ was identified in three isolates. The β-lactamases genes in the VIM-producing *K. pneumoniae* isolates were *bla*$_{CTX-M-15}$ (n=3), *bla*$_{CTX-M-14}$ (n=1), *bla*$_{SHV-1}$ (n=3), *bla*$_{SHV-12}$ (n=1), *bla*$_{TEM-1}$ (n=1) and *bla*$_{OXA-1}$ (n=1). *Aac(6')*-Ib-cr gene was confirmed in four *E. coli* and in two *K. pneumoniae* isolates. *QnrS1* was identified in two *K. pneumoniae* isolates. The class 1 integron was identified with the different gene cassette; *dfrA17-aadA5*, *dfrA5*, *dfrA12-orf-aadA2* and *dfrA17-aadA5* were identified.**

Conclusions **Our study indicated for the first time the emergence of multidrug-resistant VIM-containing *K. pneumoniae* and *E. coli* isolates of clinical origin in Gaza Strip hospitals.**

Keywords **VIM metallo-β-lactamase, CTX-M ESBL, integron, antimicrobial resistance, carbapenem, Palestine**

Introduction

Carbapenems are a β-lactam class of antibiotics used as a last resort treatment for infections caused by multidrug-resistant (MDR) Gram-negative bacteria. These drugs are also stable in response to AmpC β-lactamases and extended-spectrum β-lactamases (ESBL).[1] Resistance to carbapenems has been reported in many regions and the rate of carbapenem resistance is rising.[1] Carbapenems remain the

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treatment of choice for bacterial infections caused by ESBL-producing organisms in Palestine.[2]

In the last decade, two main groups of broad-spectrum β-lactamases have emerged in Gram-negative bacteria: the ESBLs, more commonly demonstrated in the family of Enterobacteriaceae; and the metallo-β-lactamases (MBLs), which are most frequently observed in non-fermenting Gram-negatives.[1]

Beta-lactamase enzymes that hydrolyze the β-lactam ring in β-lactam group of antibiotics are the primary resistance mechanism. Beta-lactamases are classified into four classes; the active-site serine β-lactamases (classes A, C and D) and metallo-β-lactamases (MBLs; class B), which are zinc-dependent. The enzyme groups involve CTX-M, SHV, TEM and KPC (class A); VIM, IMP and NDM (class B); and class C (CMY and ADC). Class D involves oxacillinase (OXA).[3]

Class B (MBLs) consists of imipenemase (IMP) type, German imipenemase (GIM-1), SPM-1 and VIM types along with the NDM-1. The MBL enzymes can hydrolyze all β-lactams members from all classes except for aztreonam. The activity of MBL enzymes is inhibited by EDTA. MBL genes are often carried by mobile gene cassettes inserted into class 1 integrons.[1]

Single reports and outbreaks of MBL enzymes have been detected in several countries. The NDM was reported in Palestine and Saudi Arabia.[4,5] The *Klebsiella pneumoniae* carbapenemase (KPC-2) was documented in *K. pneumoniae* and *E. cloacae* isolates in Palestine.[6] VIM-2 and VIM-4 have recently been described in *P. aeruginosa* for the first time in Palestine.[4] The first report of VIM-4 in *K. pneumoniae* was presented in a recent study in Tunisia.[7] Interestingly, another recent study reported VIM-4-positive *E. coli* isolates recovered from patients from Kuwait.[8] VIM-28 was demonstrated in Egypt and Saudi Arabia in *P. aeruginosa* isolates.[9,10]

There is a dearth of information on MBL producing *E. coli* and *K. pneumoniae* in the Middle East area, especially in Palestine. This is the first report of *E. coli* and *K. pneumoniae* clinical isolates producing VIM-4 and VIM-28 in Palestine. The aim of our study was to determine the prevalence of carbapenem-resistant isolates in hospitals in the Gaza Strip, Palestine and further to characterize these isolates.

Methods

Sample and data collection

This study was conducted for three months (April 2013 to June 2013) at three Palestinian hospitals in Gaza (Balsam Hospital, Dar Al-Shifa Hospital, and AL-Remal Health Center). All *E. coli* and *K. pneumoniae* isolates from clinical specimens were considered; these isolates were obtained from patients who were already hospitalized in Balsam Hospital and Al-Shifa Hospital but the isolates were collected from AL-Remal Health Center mostly from the outpatient department.

A total of 96 isolates (69 *E. coli* and 27 *K. pneumoniae*) were obtained from April to June 2013. The clinical specimens collected were urine, wound swabs, blood and ear discharges. Only one isolate and one specimen per patient were processed. The samples were obtained before the patients had been treated. The isolates were transferred to Laboratoire des Microorganismes et Biomolécules Actives - Tunis for the rest of the work.

The identification of the isolates was performed by API 20E systems (BioMérieux, Marcy-l'Étoile, France) to identify bacterial isolates at the species level. The *E. coli* isolates were confirmed by PCR of the *uidA* gene (F,5'ATCACCGTGGTGACGCATGTCGC3'; R,5'CACCACGATGCCATGTTCATCTGC-3').[11] The confirmation of *K. pneumoniae* isolates was based on the amplification and sequencing of *16S rRNA* gene.

Antimicrobial susceptibility testing

Susceptibility testing to 15 antibiotics was performed for all isolates by the Kirby-Bauer test according to the CLSI recommendations,[12] using antibiotic disc panels comprising: cefoxitin, ceftazidime, ampicillin, cefotaxime, amikacin, tobramycin, amoxicillin-clavulanic acid, gentamicin, nalidixic acid, ciprofloxacin, imipenem, kanamycin, trimethoprim-

sulfamethoxazole, tetracycline and chloramphenicol. The minimal inhibitory concentrations (MICs) of imipenem were tested by the agar dilution method in nine isolates.[7]

Phenotypic detection of MBL

The double-disc synergy test of imipenem-EDTA was used to screen for MBL production in all isolates. We used an overnight culture of *K. pneumoniae* and *E. coli*, which was inoculated on a plate of Mueller-Hinton agar. After drying, a blank filter paper disk with 10 µL of 0.5 M EDTA solution and a 10 µg imipenem disk were placed 10 mm apart from edge to edge. After overnight incubation, the EDTA-synergy test was positive by the presence of an enlarged zone of inhibition.[13]

Detection and characterization of β-lactamases

PCR amplification was performed for the detection of MBL-genes (bla_{IMP}, bla_{VIM}, $bla_{SPM,}$ bla_{SIM} and bla_{GIM}),[4] ESBL-genes ($bla_{OXA,}$ bla_{TEM}, bla_{CTX-M} and bla_{SHV}) and $bla_{KPC-1,2}$. Positive PCR products were sequenced and the sequences were blasted in the database of GenBank to confirm the type of β-lactamase genes (Table 1).[8,11] The carbapenem-resistance genes were assessed in the Ambedkar Centre for Biomedical Research (ACBR) – India.

The bacterial genomic DNA was used as the template, the amplification of PCR was carried out for the searched gene in a final volume of 25 µL reaction. The PCR program consisted of three steps, the denaturation: 94°C for 5 min followed by 35 cycles of 94°C for 30 s, annealing at 30 s at specific temperature (Table 1) and the extension step at 72°C for 1 min and the final extension at 72°C for 5 min. The PCR products fragments were separated on 1.5% agarose gel by agarose gel electrophoresis.

Detection of antimicrobial resistance to non-β-lactam agents

The presence of antibiotic resistance genes for sulfamethoxazole (*sul*1, *sul*3 and *sul*2), tetracycline [*tet*(A), *tet*(B), and *tet*(C)] and gentamicin [*aac*(3)-IV, *aac*(3)-I, and *aac*(3)-II] was identified by PCR.[11] The resistance genes for quinolones [*qnrA*, *qnrS*, *qnrB*, *aac*(6')-1b, and *qepA*] were also investigated in the isolates by PCR and further sequenced to recognize the variants. The presence of integron 1 was examined by PCR using the primer: (F: 5'GGGTCAAGGATCTGGATTTCG3'; R: 5'ACATGCGTGTAAATCATCGTCG3'). The *qacEΔ1-sul1* genes in the 3′-conserved segment of integron were examined in the *intI1*-positive isolates. The variable region of integron was detected by PCR and sequencing.[11]

PFGE analysis

The clonal relationship between MBL-producing isolates was identified by PFGE using the restriction enzyme *XbaI* as previously described.[8] Band pattern was compared by visual analysis by using GelCompar II program (version 6.5 Applied Maths, Ghent, Belgium).

Results

Antibiotic resistance and phenotypic detection of MBL

All 27 *K. pneumoniae* and 69 *E. coli* isolates were tested for their susceptibility towards the selected antibiotic agents. We found that 4 *E. coli* (5.8 %) and 5 *K. pneumoniae* (18.5 %) isolates were imipenem-resistant. All isolates were resistant to cefotaxime, ampicillin, imipenem and kanamycin. The resistance rate for ceftazidime, sulfamethoxazole/trimethoprim and tobramycin was quite high, at 88.9%. Resistance to amoxicillin-clavulanic acid, ciprofloxacin, chloramphenicol, gentamicin, cefoxitin and nalidixic acid among our isolates was 77.8%. However, we recorded lower resistance towards amikacin and tetracycline, 55.6%. The *K. pneumoniae* isolates (K457 and K894) exhibited high MIC values for imipenem (64 µg/mL). The total of nine carbapenem-resistant isolates showed an enhanced inhibition zone around the EDTA-IMP disk, indicating the production of metallo β-lactamases.

Detection of β-lactamase

The identification of β-lactamase revealed the presence of the bla_{VIM-4} gene in four isolates of *E.*

Table 1. PCR primers used for the analysis of the β-lactamase genes

Target gene	Sequence (5' to 3')	Annealing temperature (°C)	Amplicon size (bp)	References
bla_{VIM}	F: GATGGTGTTTGGTCGCATA R: CGAATGCGCAGCACCAG	60	390 bp	[4]
bla_{SIM}	F: TACAAGGGATTCGGCATCG R: TAATGGCCTGTTCCCATGTG	52	570 bp	[23]
bla_{IMP}	F: GGAATAGAGTGGCTTAAYTCTC R: CCAAACYACTASGTTATCT	56	188 bp	[4]
bla_{SPM}	F: AAAATCTGGGTACGCAAACG R: ACATTATCCGCTGGAACAGG	52	271 bp	[23]
bla_{GIM}	F: TCGACACACCTTGGTCTGAA R: AACTTCCAACTTTGCCATGC	52	477 bp	[4]
bla_{KPC-1}	F: AGCCGTTACAGCCTCTGGAG R: GATGGGATTGCGTCAGTTCAG	60	1351 bp	Designed for this study
bla_{KPC-2}	F: CACTGTATCGCGGTCTAGTTC R: TGTGCTTGTCATCCTTGTTAG	60	812 bp	Designed for this study
bla_{CTX-M}	F: GTTACAATGTGTGAGAAGCAG R: CCGTTTCCGCTATTACAAAC	55	1049 bp	[11]
bla_{SHV}	F: CACTCAAGGATGTATTGTG R: TTAGCGTTGCCAGTGCTCG	52	885 bp	[11]
bla_{TEM}	F: ATTCTTGAAGACGAAAGGGC R: ACGCTCAGTGGAACGAAAAC	60	1150 bp	[11]
bla_{OXA-1}	F: ACACAATACATATCAACTTCGC R: AGTGTGTTTAGAATGGTGATC	61	813 bp	[11]

coli and two *K. pneumoniae* isolates; bla_{VIM-28} was detected in three *K. pneumoniae* isolates. We did not find any bla_{KPC-1} or bla_{KPC-2} producing isolates. Screening for other β-lactamase genes in the carbapenem-resistant isolates confirmed the presence of the following β-lactamase genes in the *K. pneumoniae* isolates: $bla_{CTX-M-15}$ (n=3), $bla_{CTX-M-14}$ (n=1), bla_{SHV-1} (n=3), bla_{SHV-12} (n=1), bla_{TEM-1} (n=1) and bla_{OXA-1} (n=1). In the *E. coli* isolates, $bla_{CTX-M-15}$ was found in three isolates and $bla_{CTX-M-14}$ in one isolate (Table 2 - Appendix).

Resistance to non-β-lactam agents

Other resistance genes were identified in the VIM-producing isolates: the *sul1* gene was detected in eight isolates and *aac(3)*-II was identified in seven isolates. *Tet*B and *tet*A were found in 5 isolates and 2 isolates, respectively. We found the *aac*(6')-Ib-cr gene in four *E. coli* isolates and in two *K. pneumoniae* isolates. *QnrS1* was demonstrated in two *K. pneumoniae* isolates. The class 1 integron was found in two *E. coli* isolates with the gene cassette: *dfrA17-aadA5*. Furthermore, *intI1* was detected in 3 out of 5 VIM-producing *K. pneumoniae* isolates. The gene cassettes involved in the resistance to streptomycin (*aadA2* and *aadA5*) and trimethoprim (*dfrA5*, *dfrA12* and *dfrA17*) were present in different genetic arrangements: *dfrA17-aadA5*, *dfrA12- aadA2* and *dfrA5* (Table 2 - Appendix).

Clonal relationship by PFGE

The genetic relatedness of the isolates ranged from 74% to 100%, and four distinct PFGE types were identified (Figure 1). The PFGE analysis demonstrated 3 unrelated pulsotypes among the 5 MBL-producing *K. pneumoniae* isolates. In contrast, the two K894 and K2143

isolates showed an indistinguishable PFGE pattern (Figure 2).

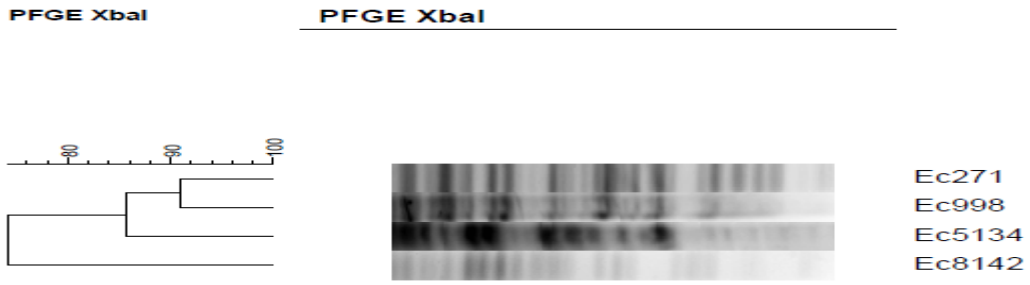

Figure 1. Pulsed-field gel electrophoresis (PFGE) dendrogram rooted from *XbaI*-digested *E. coli* strains

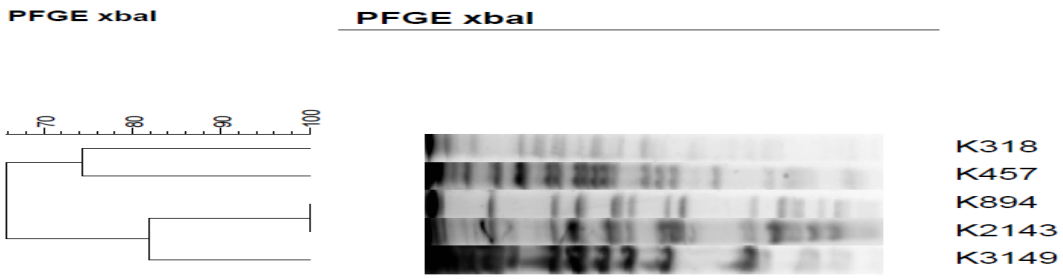

Figure 2. Pulsed-field gel electrophoresis (PFGE) dendrogram rooted from *XbaI*-digested *K. pneumoniae* strains

The percentage of 80% is used to consider an isolate as belonging to a clonal group. Therefore, the *E. coli* isolates were included in two distinct clones A and B, the clone A with three subclones (A1, A2 and A3). The *K. pneumoniae* isolates were included in three distinct clones (C, D and E) and the clone C with two subclones (C1 n=2 and C2 n=1) (Table 2 - Appendix).

Discussion

A total of 96 clinical isolates (69 *E. coli* and 27 *K. pneumoniae*) obtained from Palestinian patients in Gaza strip during a three-month period were examined for the presence of β-lactamases encoding genes, associated resistance genes and integrons.

The bla_{NDM}, bla_{KPC}, bla_{IMP} and bla_{VIM} types are more important clinically and have been detected in clinical Gram-negative bacteria particularly in the Mediterranean region and the Far East.[14] The current study showed that 18.5% of *K. pneumoniae* isolates were MBL producers. Our results are similar to a Saudian study, which showed 21.7% prevalence of MBL in *K. pneumoniae* isolates,[5] lower than that reported in Iraq (35.7%) among Gram-negative bacteria.[13]

Our findings revealed that the prevalence of MBL in *E. coli* isolates was 5.8%. Data collected from different countries showed that the MBL rate was highest among the *E. coli* strains from Iraq (45.2%).[13] In Lebanon, the MBL rate was 4% in *Acinetobacter* spp.[15] In Egypt, the MBL-producing isolates in 85% of imipenem-resistant *P. aeruginosa* were MBL-producing isolates with 29% of them harboring the bla_{VIM} gene.[16] Therefore, carbapenems can be effective treatment options in *E. coli* isolates in our hospitals, which is in agreement with the recent report.[17] One of the significant findings of this study is the resistance to carbapenem found in isolates obtained from in-patients in Balsam Hospital and Al-Shifa Hospital.

In the present study, we found bla_{VIM-4} in the isolates. In a recent study, a first occurrence of bla_{VIM-2} and bla_{VIM-4} among *P. aeruginosa* isolates in

Palestine was reported.[4] *Bla*$_{VIM-4}$ was detected among *K. pneumoniae* and *E. coli* in Kuwait.[8] A VIM-type MBL (*bla*$_{VIM-4}$) has recently been described among *K. pneumoniae* for the first time in Tunisia.[7] In Lebanon, *bla*$_{VIM-2}$ was identified in clinical *P. aeruginosa*.[18] A Jordanian study has proved the presence of *bla*$_{VIM}$, *bla*$_{IMP}$, and *bla*$_{NDM-1}$ in uropathogenic *E. coli*.[19]

The current finding of *bla*$_{VIM-28}$ is the first report in Palestine, which was demonstrated the first time in Middle East in Egypt among *P. aeruginosa* isolates.[9] Recently, this gene was reported in *P. aeruginosa* isolates in Saudi Arabia.[10] In contrast, among the MBL enzymes described in *K. pneumoniae* isolates, *bla*$_{IMP-1}$ has been detected in Lebanon.[20] The *bla*$_{GES-23}$ and *bla*$_{KPC-2}$ genes were documented in *K. pneumoniae* and *E. cloacae* isolates from rectal samples.[6]

Recently, the prevalence of MBL in *E. cloacae* isolates was 14% among different hospitals in West Bank. They have found *bla*$_{IMP}$, *bla*$_{SPM}$, *bla*$_{IMP}$ and *bla*$_{SIM}$.[21] In the Gaza strip, the presence of *bla*$_{OXA-48}$ was identified for the first time in urine in *Proteus mirabilis*.[22] The emergence of *bla*$_{VIM-4}$ in our hospitals will pose challenges for the treatment of Gram-negative bacterial infections.

The simultaneous production of three β-lactamase types (VIM-4, -28, CTX-M-14, -15, and SHV) in *K. pneumoniae* isolates is remarkable. The existence of two genes, an ESBL and MBL, in the same isolate has been reported in Enterobacteriaceae, with both CTX-M and VIM-1,[23] and VIM-4 and CTX-M-15.[7] The coexistence of MBLs and ESBLs in the same bacteria shows the important resistance genes; it is worrying since it could predict the spread and generation of MDR strains and the consequent treatment failure that can lead to significant mortality and morbidity.

In our study, class I integron was detected in *bla*$_{VIM}$-producing isolates with various arrangements of gene cassettes (*aadA2* and *aadA5*) and (*dfrA5*, *dfrA12* and *dfrA17*). The presence of the integron could increase the risk of spread and linkage to resistance genes. In Greece, the MBL was documented in an *E. coli* isolate that carried *bla*$_{VIM-1}$ with *aac*A7, *aad*A and *dhfrI* in a class I integron.[24]

In this study, all MBL-producing strains exhibited the multidrug resistance pattern along with the resistance to most non-β-lactams and β-lactams and this could be demonstrated by the fact that the β-lactamase isolates often carry an MDR-plasmid, and these carry resistance genes of non-β-lactam and β-lactam antibiotics.[8] These results highlight the alarming finding of MDR isolates producing MBL and ESBL.

The two K894 and K2143 isolates that belonged to the same clonal group and showed an indistinguishable PFGE pattern, were obtained from the same hospital (Al-Shifa), the same ward (Burn Unit) and the same week.

In our isolates, β-lactamases (*bla*$_{VIM}$ and *bla*$_{CTX-M}$) were found with *qnrS1* and *aac*(6′)-Ib-cr, a similar structure has been identified.[25]

Our data focus on the emergence of isolates producing both CTX-M-ESBL and VIM-MBL transferable enzymes in Palestine and highlight the importance of detecting the association with other resistance agents and focusing on their MDR patterns. The presence of MBLs with ESBLs in the clinical isolates of Enterobacteriaceae is a significant and emerging resistance concern due to a potential consequent failure of antibiotic therapy that can lead to significant mortality and morbidity.

Conclusions

This is the first report of *E. coli* and *K. pneumoniae* isolates producing VIM-4 and VIM-28 in Palestine. The occurrence of infections caused by carbapenem-resistant isolates in Palestine is alarming, since it endangers treatment with carbapenems; this class of antibiotics still represents a major therapeutic option for these infections, which are highly prevalent in most Palestinian hospitals. Our study highlighted the possible risk of the dissemination of multi-resistant bacteria and showed a linkage between *bla*$_{VIM}$ and *bla*$_{CTX-M}$ in the presence of *qnrS1* and *aac*(6′)-Ib-cr. This data reveals the necessity for a regular screening to detect MBL and ESBL-positive isolates circulating in Gaza hospitals, which can be an effective step in treating the infections.

Authors' contributions statement: GT designed the study, performed the experimental work (the microbiological and molecular tests), collected the data, analyzed and interpreted the data and drafted the manuscript. DN helped in performing the experimental part and interpreting the molecular part. RBS, VV and SC helped in performing the experimental part of the manuscript. AB participated in study design. MY supervised the study and contributed to final writing and editing the manuscript. KBS designed the study and supervised the study. All authors read and approved the final version of the manuscript.

Conflicts of interest: All authors – none to declare.

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Appendix

Table 2. Characteristics of the VIM-producing isolates detected in clinical samples

Bacterial code	PFGE	Antibiotics resistance	MIC of imipenem (mg/L)	Isolate	Hospital	Sample origin	MBL genes	β-lactamase genes	Non β-lactamase genes	Class 1 integron	
										intI1/qacEΔ1 +sul1	Integron structure
K318	D	AMC, CAZ, CTX, GM, AMP, IMP, KAN, AMK, SXT, FOX, TOB, CHL	16	*K. pneumoniae*	Al-Shifa	Wound	bla_{VIM-4}	$bla_{CTX-M-15}$, bla_{SHV-12}, bla_{OXA-1}	*aac (3) II, Sul1*	*IntI-1, QacSul1*	*dfrA5*
K457	E	CAZ, CTX, AMP, IMP, KAN, NAL, AMK, SXT, TOB, CIP	64	*K. pneumoniae*	Al-Shifa	Urine	bla_{VIM-4}	$bla_{CTX-M-15}$, bla_{TEM-1}, bla_{SHV-1}	*aac(6')*-Ib-cr, *Sul1*	*IntI-1,* QacSul1	*dfrA12-aadA2*
K894	C1	AMC, CAZ, CTX, GM, AMP, IMP, KAN, NAL, SXT, FOX, TOB, CHL, CIP, TET	64	*K. pneumoniae*	Al-Shifa	Wound	bla_{VIM-28}	$bla_{CTX-M-14}$	*aac (3) II, qnrS1, Sul1, TetA, TetB*	*IntI-1, QacSul1*	*dfrA17-aadA5*
K2143	C1	AMC, CAZ, CTX, GM, AMP, IMP, KAN, NAL, AMK, SXT, FOX, TOB, CHL, CIP, TET	32	*K. pneumoniae*	Al-Shifa	Wound	bla_{VIM-28}	$bla_{CTX-M-15}$, bla_{SHV-1}	*aac (3) II, aac(6')*-Ib-cr, *qnrS1, Sul1, TetB*	-	-
K3149	C2	AMC, CAZ, CTX, AMP, IMP, KAN, FOX	8	*K. pneumoniae*	Balsam	Urine	bla_{VIM-28}	bla_{SHV-1}	-	-	-
Ec271	A1	AMC, CAZ, CTX, IMP, GM, AMP, KAN, NAL, SXT, FOX, TOB, CHL, CIP, TET	32	*E. coli*	Al-Shifa	Urine	bla_{VIM-4}	$bla_{CTX-M-15}$	*aac (3) II, aac(6')*-Ib-cr, *Sul1, Sul3, TetA, TetB*	*IntI-1, QacSul1*	*dfrA17-aadA5*
Ec998	A2	AMC, CAZ, CTX, IMP, GM, AMP, KAN, NAL, SXT, FOX, TOB, CHL, CIP, TET	16	*E. coli*	Al-Shifa	Urine	bla_{VIM-4}	$bla_{CTX-M-15}$	*aac (3) II, aac(6')*-Ib-cr, *Sul1, TetB*	*IntI-1, QacSul1*	*dfrA17-aadA5*
Ec5134	A3	CTX, IMP, GM, AMP, KAN, NAL, AMK, SXT, TOB, CHL, CIP	8	*E. coli*	Balsam	Urine	bla_{VIM-4}	$bla_{CTX-M-14}$	*aac (3) II, aac(6')*-Ib-cr, *Sul1*	-	-
Ec8142	B	AMC, CAZ, CTX, GM, AMP, IMP, KAN, NAL, AMK, SXT, FOX, TOB, CHL, CIP, TET	4	*E. coli*	Al-Shifa	Wound	bla_{VIM-4}	$bla_{CTX-M-14}$	*aac (3) II, aac(6')*-Ib-cr, *Sul1, TetB*	-	-

AMC – amoxicillin–clavulanic acid; **AMK** – amikacin; **AMP** – ampicillin; **CAZ** – ceftazidime; **CHL** – chloramphenicol; **CIP** – ciprofloxacin; **CTX** – cefotaxime; **FOX** – cefoxitin; **GM** – gentamicin; **IMP** – imipenem; **KAN** – kanamycin; **NAL** – nalidixic acid; **SXT** – sulfamethoxazole/trimethoprim; **TET** – tetracycline; **TOB** – tobramycin.