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Detection of the AcrAB Efflux Pump Gene in Multidrug-Resistant Gram-Negative Bacilli Isolated from Infected Wounds at a Tertiary Care Hospital in Syria

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ABSTRACT: Multidrug-resistant Gram-negative bacilli are a growing public health threat in nosocomial settings such as surgical departments, and strains carrying the AcrAB efflux pump gene are of particular concern because this pump extrudes several unrelated classes of antibiotics. This study set out to identify the Gram-negative bacilli responsible for wound infections in a General Surgery Department, to determine their antibiotic susceptibility, and to measure the prevalence of the AcrAB gene in both chromosomal and plasmid DNA. Fifty patients admitted to a tertiary care hospital in Damascus were enrolled, and swabs were taken from the site of infection. Isolates were identified by real-time PCR, susceptibility was determined by disk diffusion according to CLSI M100, and the AcrAB gene was sought by real-time PCR with SYBR Green. Sixty-seven Gram-negative isolates were recovered, and 17 specimens (34%) yielded two species. Klebsiella pneumoniae predominated (34.33%), followed by Escherichia coli (28.36%), Enterobacter spp. (19.40%), Pseudomonas aeruginosa (13.43%) and Acinetobacter baumannii (4.48%). All isolates were multidrug-resistant. The AcrAB gene was detected in the chromosomal DNA of 67.16% and in the plasmids of 73.13% of the isolates, the plasmid figure exceeding the chromosomal one in every species examined. Isolates carrying the gene on a plasmid were resistant to aztreonam, ciprofloxacin and cotrimoxazole in 97.96% of cases and to meropenem in 91.84%, while colistin and tigecycline retained the greatest activity. The high prevalence of AcrAB on plasmids indicates a potential for horizontal transfer between strains and calls for targeted surveillance and rational antibiotic use.

1. INTRODUCTION

A wound represents a breach in the skin, and when it is accompanied by exposure of the subcutaneous tissues it creates an excellent environment that allows colonization by microorganisms and their subsequent growth, thereby causing wound infections. These hospital-acquired infections constitute a health and economic burden for patients and healthcare centers alike, since they raise the cost of treatment and prolong hospital stay, and may lead to serious conditions such as sepsis [1,2].

Wound infections are major public health issues and leading causes of morbidity and mortality, particularly in developing countries [3]. They are often polymicrobial, involving both Gram-positive cocci and Gram-negative bacilli, most commonly *Enterobacter*, *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus* spp., *Acinetobacter* and *Pseudomonas aeruginosa* [1,4].

The Centers for Disease Control and Prevention (CDC) has reported a global rise in infections caused by multidrug-resistant (MDR) pathogens, posing a significant threat to healthcare systems and contributing to the failure of antibiotic therapy [5,6]. A bacterial strain is classified as MDR when it demonstrates resistance to at least one agent in three or more different antibiotic

classes [7]. Variation in the bacterial species associated with wound and burn infections, and in their antibiotic resistance patterns, can significantly influence treatment strategies and the therapeutic options available [8].

The resistance that bacteria display towards the various antibiotics may be intrinsic, arising from chromosomal genes, or acquired, resulting either from genetic mutations following exposure to antibiotics or from the acquisition of resistance determinants from other bacteria of the same or of different species through horizontal gene transfer (HGT). This transfer may occur by one of three mechanisms — conjugation, transformation and transduction — all of which ultimately drive the emergence of multidrug resistance across species [9,10,11].

Bacteria employ several mechanisms to resist antibiotics. Some species reduce the penetration of antibiotic molecules to their active sites within the bacterial cell, or modify the structure of the cellular target, while others extrude the antibiotic molecules from the cell by means of efflux pumps [12]. The action of these pumps lowers the intracellular concentration of the antibiotic and thereby allows the bacterium to tolerate high concentrations of it [13].

Six families of efflux pumps can be distinguished, classified according to their ability to extrude different classes of antimicrobial agents. The great majority of the active efflux pumps in Gram-negative bacteria belong to the RND family, which is the most efficient of all pump types. These pumps play a broad role in antimicrobial resistance by conferring resistance to numerous antibiotics, including tetracyclines, fluoroquinolones, aminoglycosides and penicillins [14].

The AcrAB−TolC pump is the most extensively studied of the RND pumps and is known for its capacity to extrude a wide variety of antibiotics, thereby producing multidrug resistance [15]. This mechanism is considered important for the acquisition of antibiotic resistance in Gram-negative bacilli such as *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*. Drug efflux is further reinforced by the presence of active efflux pump genes, such as those of the AcrAB-TolC system, on plasmids and other mobile genetic elements, which promotes the horizontal transfer of these genes and increases the dissemination of resistance in clinical settings [16,17,18].

Studies have shown that the presence of the AcrAB gene contributes substantially to multidrug resistance. This tripartite system actively extrudes a broad range of antibiotics, including β-lactams, fluoroquinolones, tetracyclines and chloramphenicol, thereby reducing their intracellular concentrations and consequently their efficacy [19].

2. AIM OF THE STUDY

The study aims to identify the multidrug-resistant Gram-negative bacilli isolated from general surgery patients, to examine the prevalence of the AcrAB efflux pump gene among these organisms, and to determine their antibiotic susceptibility, so that measures may be taken to control the infections they cause and to limit the development of high-level antibiotic resistance in these strains.

3. MATERIALS AND METHODS

Study design: A cross-sectional study.

Samples: The samples comprised the infected wound sites of 50 patients admitted to the General Surgery Department of a tertiary care hospital in Damascus who had undergone various surgical procedures between the beginning of May 2026 and the end of July 2026.

Specimens were taken with a sterile cotton swab from the affected site in patients presenting with signs or symptoms of infection. Immediately after collection, each swab was placed in a sterile liquid preservation medium (xanthan gum / L-cysteine / sodium thioglycolate / peptone) [20] pending transport to the laboratory for culture and further testing.

1. Isolation and identification of the bacteria

The presence of Gram-negative bacilli in the specimen was established by preparing a smear and staining it with Gram stain, culturing the specimen on media selective for Gram-negative bacilli, and determining its biochemical characteristics.

EMB agar and cetrimide agar (Himedia Laboratories, India) were used for culture, and the media were prepared according to the manufacturer's instructions. Each specimen was streaked onto both media and then incubated at 37 °C for 24 hours, allowing single colonies to be obtained.

An oxidase test was performed, a positive result indicating the presence of *Pseudomonas aeruginosa* in the specimen. The following biochemical tests were also carried out: indole, methyl red, Voges-Proskauer and citrate, together with culture on Kligler iron agar, in order to determine the species of the bacterial isolates.

The isolates were additionally typed by molecular testing. Bacterial DNA was extracted from the specimens using an extraction kit (GeneAll® Exgene™ Cell SV mini, South Korea) according to the manufacturer's instructions, and the species was then determined by real-time polymerase chain reaction (Real Time-PCR) using species-specific kits (Genetic PCR Solutions™, Genetic Analysis Strategies, Spain).

The reaction mixture was prepared in nuclease-free tubes to a final volume of 20 µl as shown in Table 1.

Table 1. Composition of the reaction mixture for a 20 µl final volume

Reaction component	Volume (µl)
Nuclease-free water	9
Reaction mix	5
Primer and probe mix	1
Extracted DNA	5

Thermal amplification was carried out according to the manufacturer's instructions for the species-specific kits, as set out in Table 2.

Table 2. Thermal amplification programme for isolate typing

Cycles	Step	Temperature	Time
1	Initial denaturation	95 °C	2 min
40	Denaturation	95 °C	5 s
40	Annealing and extension	60 °C	20 s

2. Antibiotic susceptibility testing

Susceptibility testing of the isolated strains against several classes of antibiotics was performed by the disk diffusion method (Kirby–Bauer method) on Mueller Hinton agar, in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI) M100, 33rd edition [21]. Disks impregnated with defined concentrations of antibiotics (Biomaxima, Poland) were used at the following concentrations: amikacin (30 µg), aztreonam (30 µg), ampicillin/sulbactam (20 µg), cefepime (30 µg), cefoperazone (75 µg), ceftazidime (30 µg), chloramphenicol (30 µg), ciprofloxacin (30 µg), colistin (10 µg), cotrimoxazole (25 µg), doxycycline (30 µg), gentamicin (10 µg), imipenem (10 µg), meropenem (10 µg) and tigecycline (15 µg).

A bacterial suspension was prepared from colonies of each isolated strain to a turbidity matching the 0.5 McFarland standard. Fifty microlitres of the suspension were spread over the entire surface of the Mueller Hinton agar in a Petri dish, the antibiotic-impregnated disks were applied to the medium, and the plates were then incubated at 37 °C for 24 hours.

Results were read after incubation by measuring the diameter of the inhibition zones around each disk and comparing them with the standard tables in order to determine the resistance of the organism to the various antibiotics.

3. Detection of the AcrAB efflux pump gene

The AcrAB gene was sought in both the chromosomal (genomic) DNA and the plasmid DNA of the isolates that had proved multidrug-resistant in the susceptibility testing described above. Genomic and plasmid DNA were extracted from each isolate using the GeneAll® Exgene™ Cell SV mini (South Korea) and GeneAll® Exprep™ Plasmid SV mini kits according to the manufacturer's instructions.

Detection of the AcrAB gene in the genomic and plasmid DNA was carried out by real-time polymerase chain reaction (RT-PCR) using specific primers (F: ATCAGCGGCCGGATTGGTAAA; R: CGGGTTCGGGAAAATAGCGCG) supplied by Genscript (Singapore) [22].

GeneAll® RealAmp™ 2X SYBR qPCR Master mix was used to prepare the reaction mixture to a final volume of 20 µl as shown in Table 3.

Table 3. Composition of the reaction mixture for a 20 µl final volume

Component	Concentration	Volume
2X SYBR qPCR Master mix	2X	10 µl
Template DNA	(10 pg–10 ng)/µl	—
Forward primer	100 µM	0.1 µl
Reverse primer	100 µM	0.1 µl
Nuclease-free water	—	to 20 µl

Thermal amplification was carried out as shown in Table 4.

Table 4. Thermal amplification programme for detection of the AcrAB gene

Step	Temperature	Time	Cycles
Enzyme activation	95 °C	15 min	1
Denaturation	95 °C	20 s	40
Annealing	59 °C	40 s	40
Extension	72 °C	30 s	40

4. RESULTS

1. Isolation and identification of the bacteria

Culture of the specimens taken from the 50 patients yielded 67 Gram-negative isolates. Molecular typing by real-time PCR identified these isolates, and Figure 1 shows the amplification curves obtained for each of the Gram-negative species isolated from the specimens.

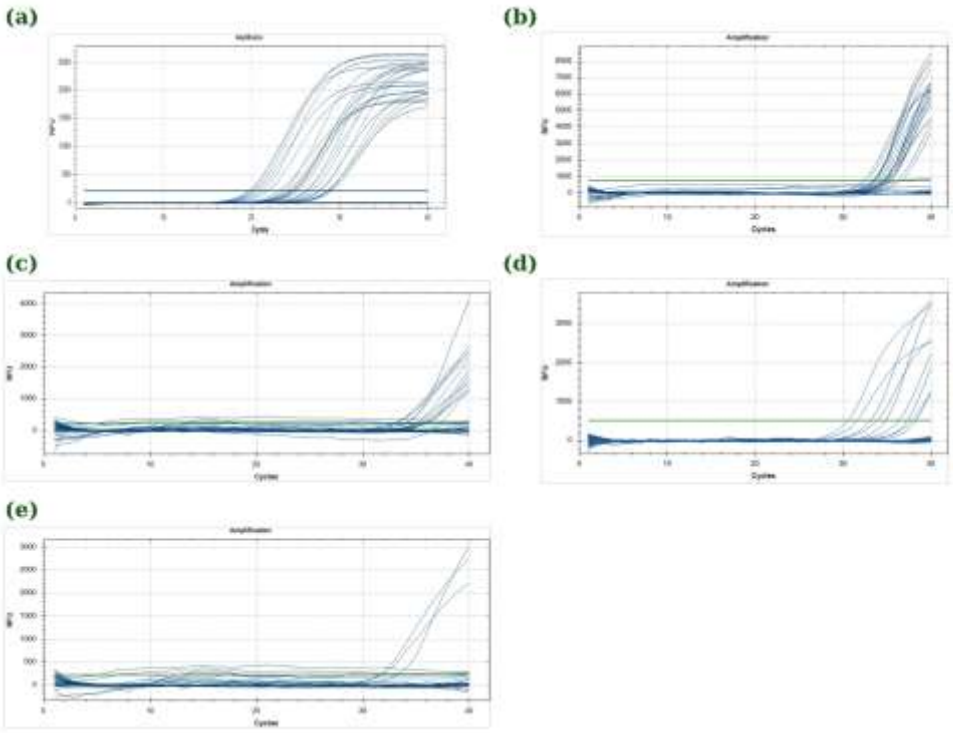

Figure 1. Gene amplification curves for each bacterial species in the specimens:

(a) *Klebsiella pneumoniae*, (b) *Escherichia coli*, (c) *Enterobacter* spp., (d) *Pseudomonas aeruginosa*, (e) *Acinetobacter baumannii*

Seventeen specimens (34%) contained two species of Gram-negative bacilli, while the remaining 33 specimens (66%) contained only one.

Twenty-three isolates of *Klebsiella pneumoniae* were recovered, accounting for 34.33% of all isolates and thus the highest proportion among the Gram-negative bacilli. Nineteen isolates of *Escherichia coli*, 13 of *Enterobacter* spp. and 9 of *Pseudomonas aeruginosa* were recovered, representing 28.36%, 19.40% and 13.43% of the total respectively, whereas only 3 isolates of *Acinetobacter baumannii* were obtained, representing 4.48% of all isolates, as shown in Figure 2.

Male patients accounted for 74% and female patients for 26% of those enrolled in the study.

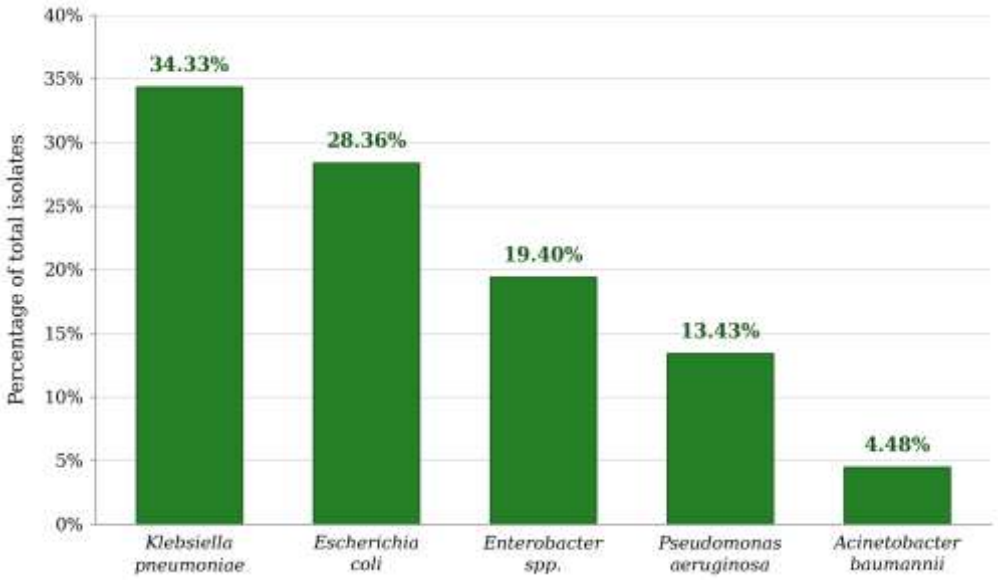

Figure 2. Percentage distribution of the Gram-negative bacilli species in the specimens

2. Antibiotic susceptibility results

Disk diffusion testing showed that every strain isolated from the specimens was resistant to at least four antibiotics belonging to different classes; all the isolated organisms were therefore multidrug-resistant (MDR) strains. Table 5 presents the susceptibility and resistance of the isolates to the various antibiotics.

Table 5. Susceptibility and resistance of all isolated strains to the various antibiotics

Antibiotic	*Klebsiella*(n=23)			*E. coli*(n=19)			*Enterobacter*(n=13)			*Pseudomonas*(n=9)			*Acinetobacter*(n=3)		
	S%	I%	R%	S%	I%	R%	S%	I%	R%	S%	I%	R%	S%	I%	R%
AK 30	13.04	8.70	78.26	31.58	26.32	42.11	15.38	23.08	61.54	11.11	0	88.89	0	33.33	66.67
SAM 20	4.35	4.35	91.30	26.32	5.26	68.42	0	7.69	92.31	0	0	100	0	0	100
ATM 30	8.70	0	91.30	10.53	0	89.47	0	0	100	0	0	100	0	0	100
FEP 30	8.70	0	91.30	0	5.26	94.74	0	30.77	69.23	0	11.11	88.89	0	0	100
CEP 75	4.35	4.35	91.30	5.26	0	94.74	15.38	15.38	69.23	11.11	0	88.89	0	0	100
CAZ 10	4.35	0	95.65	5.26	0	94.74	0	0	100	0	11.11	88.89	0	33.33	66.67

Antibiotic	*Klebsiella*(n=23)			*E. coli*(n=19)			*Enterobacter*(n=13)			*Pseudomonas*(n=9)			*Acinetobacter*(n=3)		
C 30	8.70	17.39	73.91	21.05	10.53	68.42	15.38	30.77	53.85	11.11	22.22	66.67	0	0	100
CIP 30	4.35	4.35	91.30	15.79	5.26	78.95	15.38	7.69	76.92	11.11	0	88.89	0	0	100
CT 10	65.22	0	34.78	68.42	0	31.58	61.54	0	38.46	55.56	0	44.44	66.67	0	33.33
SXT 25	4.35	0	95.65	26.32	5.26	68.42	15.38	0	84.62	0	0	100	0	0	100
DOX 30	21.74	21.74	56.52	52.63	0	47.37	30.77	30.77	38.46	22.22	11.11	66.67	0	33.33	66.67
GEN 10	26.09	8.70	65.22	36.84	15.79	47.37	30.77	7.69	61.54	11.11	0	88.89	0	33.33	66.67
IMP 10	21.74	4.35	73.91	68.42	0	31.58	38.46	15.38	46.15	0	11.11	88.89	0	33.33	66.67
MEM 10	8.70	8.70	82.61	10.53	15.79	73.68	30.77	0	69.23	11.11	0	88.89	0	0	100
TGC 15	34.78	21.74	43.48	68.42	21.05	10.53	76.92	15.38	7.69	0	0	100	0	0	100

S: susceptible; I: intermediate; R: resistant. AK: amikacin; SAM: ampicillin/sulbactam; ATM: aztreonam; FEP: cefepime; CEP: cefoperazone; CAZ: ceftazidime; C: chloramphenicol; CIP: ciprofloxacin; CT: colistin; SXT: cotrimoxazole; DOX: doxycycline; GEN: gentamicin; IMP: imipenem; MEM: meropenem; TGC: tigecycline.

3. Detection of the AcrAB gene

Thermal amplification by real-time PCR with SYBR Green fluorescent dye demonstrated the AcrAB gene in the chromosomal (genomic) DNA of 45 isolates, representing 67.16%, and in the plasmid DNA of 49 isolates, representing 73.13% of all isolates.

The gene was present in the genome of 17 *Klebsiella pneumoniae* isolates (73.91%) and in the plasmids of 19 isolates (82.61%). Among the *Escherichia coli* isolates it was detected in the genome and the plasmids of 10 isolates, 52.63% in each case. In *Enterobacter* spp. the AcrAB gene was present in the genome of 8 isolates (61.54%) and in the plasmids of 9 isolates (69.23%). The gene was also found in the genome of 7 *Pseudomonas aeruginosa* isolates (77.78%) and in the plasmids of 8 of them (88.89%), and in both the genome and the plasmids of all three *Acinetobacter baumannii* isolates (100% in each case), as set out in Table 6.

Figure 3 shows the amplification curves indicating the presence of the AcrAB gene in the multidrug-resistant Gram-negative bacilli isolated.

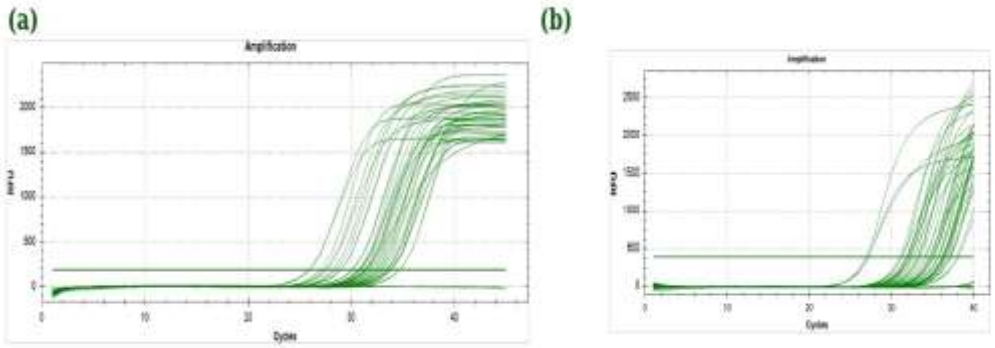

Figure 3. Amplification curves indicating the presence of the AcrAB gene in the isolates studied:

(a) in the plasmid, (b) in the chromosomal DNA

Table 6. Prevalence of the AcrAB gene in the genome and plasmids of the isolates studied

Species	AcrAB in the genome	AcrAB in the plasmid
Klebsiella pneumoniae (n=23)	73.91% (17)	82.61% (19)
Escherichia coli (n=19)	52.63% (10)	52.63% (10)
Enterobacter spp. (n=13)	61.54% (8)	69.23% (9)
Pseudomonas aeruginosa (n=9)	77.78% (7)	88.89% (8)
Acinetobacter baumannii (n=3)	100% (3)	100% (3)
Total (n=67)	**67.16% (45)**	**73.13% (49)**

Table 7 shows the resistance rates to the antibiotics studied among the Gram-negative bacilli carrying the AcrAB gene on a plasmid (49 isolates).

Table 7. Antibiotic resistance rates among the strains carrying the AcrAB gene

Antibiotic	Resistant isolates	Percentage (%)
Aztreonam	48	97.96
Ciprofloxacin	48	97.96
Cotrimoxazole	48	97.96
Ampicillin/sulbactam	47	95.92
Cefepime	47	95.92
Cefoperazone	47	95.92
Ceftazidime	47	95.92
Meropenem	45	91.84
Chloramphenicol	40	81.63
Amikacin	39	79.59
Gentamicin	38	77.55
Imipenem	36	73.47
Doxycycline	28	57.14
Tigecycline	23	46.94
Colistin	20	40.82

5. DISCUSSION

Our study showed the presence of multidrug-resistant strains in all patients with infection at the wound site.

Most of the infections in our study occurred in men (74%) rather than women (26%), a finding consistent with that of Ruegsegger et al. in North Carolina in 2022, where the infection rate was 76% in men and 24% in women [23].

The proportion of patients whose wound sites yielded more than one bacterial species was 34% in our study. This is higher than the 17.2% recorded by Ruegsegger et al. [23], similar to the 33.85% reported by El Hamzaoui et al. in Morocco in 2020 [24], and lower than the 63.5% found by Kabanangi et al. in Tanzania in 2021 [25]. The difference between these figures may be attributable to the presence of previous infections at the same site.

Distribution of the bacterial species isolated

Klebsiella pneumoniae ranked first among our isolates at 34.33%, followed by *Escherichia coli* at 28.36%, *Enterobacter* spp. at 19.40% and *Pseudomonas aeruginosa* at 13.43%, with *Acinetobacter baumannii* last at 4.48%. This ranking is not far removed from that reported by Mansoor et al. in Kashmir in 2024, a study covering 1,413 Gram-negative isolates from wound infections in which 395 isolates of *E. coli*, 326 of *Klebsiella*, 268 of *Acinetobacter* and 220 of *Pseudomonas* were recovered [26], although in that study the leading organism was *E. coli* rather than *Klebsiella*. It is notable that the proportion of *E. coli* in our study (28.36%) is almost identical to its share of the Gram-negative isolates in that study (28.0%).

Our finding that *K. pneumoniae* predominates agrees with that of Hamed et al. at Suez Canal University Hospitals in 2024, where *K. pneumoniae* was the Gram-negative organism most frequently isolated from surgical site infections, accounting for 7 of 16

Gram-negative isolates [27]. By contrast, our result differs from that reported by Badger-Emeka in 2026 for 270 Gram-negative isolates from wounds, in which *P. aeruginosa* led with 112 isolates, followed by *E. coli* with 59, *A. baumannii* with 52 and *K. pneumoniae* with 42 [28]. Likewise, El Hamzaoui et al. in Morocco found *P. aeruginosa* and *A. baumannii* ahead of *K. pneumoniae*, the first two each accounting for 15.38% and *Klebsiella* for 13.85% of the 130 isolates recovered [24].

This variation is most probably due to differences in the type of ward from which the specimens were collected. Our study was confined to general surgery patients, whereas the comparator studies covered burn units or several departments, and *P. aeruginosa* and *A. baumannii* are more closely associated with the environment of burn and intensive care units and with longer stays there. Differences in local colonization patterns and in antibiotic stewardship policies between centers also contribute. It is striking that *Enterobacter* spp. ranked third in our study at 19.40% but did not appear at all among the isolates of Mansoor et al. or Badger-Emeka [26,28]. This may reflect a difference in the typing method, since *Enterobacter* may readily be classified as *Klebsiella*, or the reverse, when biochemical tests alone are relied on — a limitation that our study avoided by using molecular typing.

Prevalence of the AcrAB gene among the isolates

The AcrAB gene was detected in the chromosomal DNA of 67.16% of our isolates and in the plasmids of 73.13%. The fact that the plasmid figure exceeds the chromosomal one is the most striking finding in this regard, because the presence of the gene on a mobile genetic element opens the way to its horizontal transfer between strains and species, and not merely between strains descended from a common ancestor. This interpretation is supported by the demonstration of Nolivos et al. in 2019 that the AcrAB-TolC system is not simply a transporter of antibiotics but also plays a part in the acquisition of resistance through plasmid transfer itself [29]. Lee et al., in a study of 101 carbapenem-resistant Enterobacterales in southern Taiwan, likewise found the *acrB* gene within class 1 integrons in 47.5% of the integron-positive isolates [30], confirming the association of this gene with mobile genetic elements.

Considering each species separately, the gene was present in 73.91% of *K. pneumoniae* genomes and 82.61% of their plasmids. These are high figures, yet they fall short of those recorded by ALmiyah in 2023 in Al-Diwaniyah Province — a study resembling ours in that it examined isolates from wound infections — where the *acrA* and *acrB* genes were each detected in all 55 multidrug-resistant *K. pneumoniae* isolates (100%) [31]. Ali and Ibrahim in 2025 reported comparable figures, with *acrA* at 100% and *acrB* at 95.08% [32]. Our figures are, by contrast, considerably higher than those of Aguilar-Luis et al. in Lima in 2026, who detected *acrB* in only 9 of 16 isolates (56.3%) and *acrA* in 7 (43.8%) [33], and higher than the 47.7% recorded by Lee et al. for *K. pneumoniae* within class 1 integrons [30].

Escherichia coli carried the gene in 52.63% of both genomes and plasmids, the lowest figure among our species. This differs markedly from the findings of Maleki et al. in Tehran in 2017, who studied 123 multidrug-resistant urinary isolates and reported *acrA* at 82.90% and *acrB* at 95.90% [34], and from those of Sabah and AL-Oqaili in 2024, who found the gene in all of their isolates [35]. Lee et al., on the other hand, recorded a very low figure for *E. coli*, not exceeding 7.1% within integrons [30]. This wide variation is most likely attributable to differences in the site of isolation and the type of specimen — wound isolates differing in their genetic background from urinary isolates — and to differences in the primers used and the region of the marker targeted.

Enterobacter isolates carried the gene in 61.54% of genomes and 69.23% of plasmids, figures close to the 68.8% recorded by Lee et al. for this genus within integrons [30]. The finding agrees in its general direction with the conclusion of Jiang et al. in 2025, who observed over-expression of the AcrAB-TolC system in NDM-1-producing *Enterobacter cloacae* complex isolates, all of which were extensively drug-resistant [36].

Finally, the detection of the gene in *P. aeruginosa* at 77.78% in the genome and 88.89% in the plasmid, and in all three *A. baumannii* isolates at 100%, requires comment. The system under study is originally an enterobacterial one, whereas the system predominant in *P. aeruginosa* is MexAB-OprM and in *A. baumannii* AdeABC. All three systems, however, belong to the RND family and share a common architecture and a high degree of similarity: Venter et al. report that the AcrB and MexB transporters share 86% similarity and that the latter can functionally substitute for the former [37], while Wilhelm and Pos describe AdeB as a homologue of AcrB that adopts the same structural fold and has nearly identical drug substrate specificity [38]. The high figures recorded for these two species may therefore reflect the amplification of conserved regions shared between homologues of the same family — a possibility that can be neither excluded nor confirmed without sequencing the amplicons. We regard this as a limitation of the present study and recommend that it be addressed in future work.

Antibiotic resistance of the isolates carrying the AcrAB gene

The 49 isolates carrying the AcrAB gene on a plasmid displayed very high resistance to most of the antibiotics studied. Resistance reached 97.96% to aztreonam, ciprofloxacin and cotrimoxazole alike, 95.92% to ampicillin/sulbactam, cefepime, cefoperazone and ceftazidime, and 91.84% to meropenem. This profile is consistent with what is known of the substrate range of this system, which extrudes β-lactams, fluoroquinolones, tetracyclines and chloramphenicol from the cell [19].

Our resistance rates exceed by a wide margin those recorded by ALmiyah in 2023 for *K. pneumoniae* isolates from wounds, even though all of her isolates carried the gene: resistance there reached 59.7% to ceftazidime, 25.2% to ciprofloxacin and 18.3% to gentamicin [31], against 95.92%, 97.96% and 77.55% respectively in our study. This difference indicates that carriage of the gene alone does not suffice to account for the level of resistance; the phenotypic effect depends on the level of expression of the system and on the other mechanisms accompanying it, particularly the production of extended-spectrum β-lactamases and carbapenemases. Support for this comes from the finding of Lee et al. that inactivation of the *acrB* gene lowered the minimum inhibitory concentration two- to sixteen-fold for fluoroquinolones, tetracyclines and tigecycline, without appreciably altering susceptibility to the carbapenems [30]. This suggests that the high meropenem resistance in our isolates (91.84%) is largely attributable to other mechanisms not addressed in this study, even though Chetri et al. have demonstrated that the system does contribute to carbapenem non-susceptibility in *E. coli* [17].

The lowest resistance rates, by contrast, were those to colistin (40.82%), tigecycline (46.94%) and doxycycline (57.14%), which therefore represent the therapeutic options retaining the greatest activity against our isolates. That colistin resistance should reach this level nonetheless warrants attention, and it cannot be ascribed to the AcrAB pump, since colistin is not among its substrates; it is more likely to arise from modifications of the lipopolysaccharide structure of the outer membrane. The intermediate position of tigecycline and doxycycline, despite both being substrates of the system, accords with the demonstration by Nasralddin et al. that exposure to tetracycline and chloramphenicol reduces susceptibility to tigecycline and induces genetic alterations in the regulators of the system in *E. coli* and *K. pneumoniae* [19] — that is, resistance to these two agents is liable to worsen under continued selective pressure.

Taken as a whole, our findings approach those of Jiang et al., for whom isolates over-expressing AcrAB-TolC were extensively drug-resistant, being resistant to carbapenems, fluoroquinolones, tetracyclines and cotrimoxazole together [36] — a pattern matching that recorded here, and one indicating that the strains carrying this gene in the General Surgery Department constitute a reservoir of broad-spectrum resistance that warrants dedicated surveillance.

6. CONCLUSIONS AND RECOMMENDATIONS

Conclusions: This study has demonstrated the spread of multidrug-resistant Gram-negative bacilli among general surgery patients at a tertiary care hospital in Damascus, and has shown the wide prevalence of the AcrAB gene among these organisms, particularly on plasmids, which facilitates its extensive transfer between species. Its presence was accompanied by high-level, multiple resistance in these strains, giving rise to considerable therapeutic difficulties and prolonging hospital stay. These patients thus become reservoirs for such strains, increasing the likelihood of transmission within the department and consequently posing a risk to other patients.

Recommendations: The results point to the importance of continuous and periodic monitoring of antibiotic resistance in Gram-negative bacilli causing wound infections, and of the early detection of strains carrying this gene in an effort to limit their spread among patients, through preventive measures including the isolation of affected patients and the selection of the most appropriate antibiotics to improve the efficacy of treatment. It is further recommended that the amplicons be sequenced to confirm the identity of the gene in the non-enterobacterial species, that the expression level of the system be measured and efflux pump inhibitors used in subsequent studies, and that extended-spectrum β-lactamases and carbapenemases be sought concurrently in order to distinguish the contribution of each mechanism to the resistance phenotype.

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AUTHOR CONTRIBUTIONS

Razan AL Debs: Conceptualization, methodology, investigation, formal analysis, writing – original draft, writing – review and editing.

Nader Ghanayem: Methodology, validation, and technical supervision of the molecular work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

The study was approved by the Research Ethics Committee of the Faculty of Pharmacy, Damascus University in 2025 (approval number SRC-1810). Informed consent was obtained from all participants, and the specimens were handled anonymously.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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