



RESEARCH ARTICLE

Antimicrobial resistance surveillance among gram-negative bacterial isolates from patients in hospitals in Khartoum State, Sudan [version 1; referees: awaiting peer review]

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Abstract

Background: Antimicrobial resistance (AMR) among gram-negative bacilli is a global health problem. Surveillance of AMR is required to advise on empirical antimicrobial therapy. This study aimed at evaluating the frequency and the AMR patterns of gram-negative isolates from patients treated in eight hospitals in Khartoum State, Sudan.

Methods: A cross-sectional laboratory-based study was conducted over a 6 months period at the Microbiology Department, Soba University Hospital-Khartoum State, Sudan. All gram-negative isolates from blood, urine, wound, and sputum during the period of study were included. Identification and antimicrobial susceptibility testing were carried out for all isolates.

Results: A total of 734 Gram-negative bacilli were isolated. *Klebsiella pneumoniae* (249 isolates, 34%) was the most frequently encountered one, followed by *Pseudomonas aeruginosa* (153 isolates, 21%), *E.coli* (123 isolates, 17%), *Acinetobacter baumannii* (75 isolates, 10%), *Burkholderia cepacia* (42 isolates, 6%), *Proteus mirabilis* and *Proteus vulgaris* (28 isolates, each, (4%) *Enterobacter colecaes* (28 isolates, 4%), *Stenotrophomonas maltophilia* (21 isolates, 2.8%), and other gram-negative bacilli (15 isolates, 2.2%) The analysis of the antimicrobial susceptibility patterns showed that 134 (22.3%) isolates were resistant to three or more classes of antibiotics, including cephalosporins, β -lactam- β -lactamase inhibitor, quinolones, aminoglycosides and carbapenems.

Conclusion: This high level of resistance among gram-negative bacilli in Khartoum state hospitals is alarming. The local health authorities should be prompted to step up infection control programs and introduce the concept of antimicrobial stewardship in Khartoum State hospitals.

Keywords

Gram-negative bacilli, Multidrug resistant bacteria, laboratory-based study, Surveillance.

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Review

Any reports and responses or comments on the article can be found at the end of the article.

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Introduction

Antimicrobial resistance (AMR) constitutes a continuously growing threat to the effective treatment of microbial infections¹. However, the direct impact of AMR on the health of hospitalized patients or the people in the community, as well as the financial burden experienced by health care systems in managing the infections and complications due to AMR, are still mostly uncertain². Antibacterial drugs are widely used worldwide both in human health and food industry. Overuse of these medications can favor the selection and the spread of multidrug resistant (MDR) bacteria¹. Multi drug resistance is defined as resistance to at least three different antibiotic groups, as reported by Masgala and Kostaki³. Antibiotic resistance among a variety of bacterial species is increasing in healthcare and community setting. Extended-spectrum β -lactamase and carbapenemase production are the most frequently emerging resistance mechanisms among gram-negative bacilli⁴. Gram-negative bacilli including Enterobacteriaceae and non-lactose fermenting bacteria such as *Pseudomonas* spp. and *Acinetobacter* spp. are the main causes of hospital-acquired infection in critical care units^{3,5}. The antibiotic resistance rates among these organisms have amplified uncontrollably in a matter of a few years to become worldwide². According to the Centre for Disease Control and Prevention, gram-negative bacilli are able to develop antibiotic resistance through multiple methods and are particularly competent at spreading the resistance among species via horizontal gene transfer⁶. The enigma of AMR is particularly plaguing low- and middle-income countries, where infectious diseases are the commonest cause of hospitalization and death; and the newer antibiotics cannot be afforded⁷.

AMR surveillance is key for determining the prevalence patterns of AMR, which is fundamental for the development of national and international treatment strategies. Most of the available surveillance data are derived from developed countries; the studies conducted in developing countries are unfortunately not adequate.

This surveillance study was undertaken in order to find out the different types of the AMR patterns of bacterial pathogens isolated from patients in Khartoum State, Sudan. This study may help in formulating antibiotic policies tailored to our hospitals. These data can be used as “information for action” antibiotic stewardship and interventions to optimize antibiotic prescribing practice, therefore prolongs the usefulness of existing antibiotics.

Methods

Study design and clinical strains

This is a cross-sectional laboratory based study carried out in the department of medical microbiology Soba University Hospital (SUH) and Institute of Endemic Diseases, University of Khartoum, Sudan. A total of 734 Gram-negative bacteria were isolated from patients treated in eight hospitals in Khartoum state including: two university hospitals (Soba university hospital and Bashaier); three teaching hospitals (Ibrahim Malik, Bahri and Sadabulalla), a specialized hospital (Elfouad), and two private hospitals (Imperial and Elswedy). This study included all clinical specimens received in Soba University hospital microbiology

laboratory in a period from October 2016 to February 2017 from various wards in the aforementioned hospitals including: intensive care units (ICUs), neonatal ICUs, medicine units, surgery units, pediatric units, and renal units. The isolates were collected from different clinical specimens including: blood (243 isolates, 33.1%), urine (230 isolates, 31.3%), wounds (183 isolates, 25%), sputum (22 isolates, 3%), catheter tips (26 isolates, 3.4%) and body fluids (including cerebrospinal, peritoneal, pleural, acetic and synovial fluid; 30 isolates 4.2%). Microorganisms were grown on Blood, Chocolate and MacConkey agar. Then, they were identified according to standard microbiological procedures (based on colony morphology, microscopy, and biochemical tests)⁸. Quality control strains were used in biochemical tests and antimicrobial susceptibility testing [*E. coli* (ATCC #25922) and *P. aeruginosa* (ATCC #27853)].

Most gram-negative Bacilli isolates were further identified and confirmed by PCR. Guanidine chloride method, as described by Alsadig *et al.*⁹, was used for DNA extraction followed by PCR which it was carried out using thermal cycler (analytikjena® Biometra TADVANCED, Germany), by using the following primers (Macrogen, Korea), using species-specific primers for *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and Universal 16S rRNA primers (Table 1) the reaction was carried out in a total reaction volume of 25 μ l (5 μ l Master mix of Maxime RT premix kit (iNtRON Biotechnology, Seongnam, Korea), 0.6 μ l of forward primer, 0.6 μ l of reverse primer, 2 μ l of DNA and 16.8 μ l deionized sterile water). The cycle condition as the following: initial denaturation step at 94°C for 5-min, followed by 30 cycles of denaturation at 94°C for 45 seconds, primer annealing temperature according to the primers Table 1 for 45 seconds, followed by step of elongation at 72°C for 60 seconds and the final elongation at 72°C for 5 min¹⁰. The purity and integrity of each PCR product was evaluated electrophoresis in a 2% agarose gel in TBE 1X, that contain 2.5 μ l of (20mg/ml) ethidium bromide at 100V for 40 min. The specific amplified product were detected by comparing with 100 base-pairs standard DNA ladder (iNtRON BIOTECHNOLOGY, Seongnam, Korea) Bands were visualized under U.V transilluminater (analytikjena® Biometra BDacom-pact, Germany). The PCR product of 16SrRNA were purified and Sanger sequencing was performed by Macrogen Company (Seoul, Korea). Then nucleotides sequences of the genes 16SrRNA achieved were searched for sequence similarity using nucleotide BLAST for species identification¹¹.

Antimicrobial susceptibility testing

Susceptibility testing was performed using the Kirby-Bauer disc-diffusion method; each isolate was swabbed on the Muller-Hinton agar and the antibiotic discs were placed on top and incubated at 37°C for 18–24 hours¹². all isolates were tested against the following antibiotic disc (Mast Diagnostic): amoxycillin clavulanate (AMC) (30 μ g), cefuroxime (CXM) (30 μ g), cephalexin (CL) (30 μ g), ceftriaxone (CRO) (30 μ g), ceftazidime (CAZ) (30 μ g), meropenem (MEM) (10 μ g), imipenem (IPM) (10 μ g), amikacin (AK) (30 μ g), gentamicin (Gen) (10 μ g), ciprofloxacin (CIP) (5 μ g), trimethoprim-sulfamethoxazole (SXT) (25 μ g), temocillin (TEM) (30 μ g), azteroname (AZT) (30 μ g) and

Table 1. Bacterial gene primers used for PCR.

Species specific primers	Primer (5' to 3')	Tm (°C)	Band size (bp)	Ref
Universal 16S rRNA	F=AGAGTTTGATCCTGGCTCAG	58	890	10
	R=CTACGGCTACCTTGTACGA	58		
<i>K. pneumoniae</i>	Pf = ATTTGAAGAGGTTGCAAACGAT	56		13
	Pr1=TTCACTCTGAAGTTTCTTGTTTC	56	130	
	Pr2 = CCG AAG ATG TTT CAC TTC TGA TT	56	260	
<i>E.coli</i>	ECA75F=GGAAGAAGCTTGCTTCTTTGCTGAC	52	544	14
	ECR619R=AGCCCGGGGATTTCACATCTGACTTA	52		
<i>Pseudomonas spp.</i>	F= GGGGGATCTTCGGACCTCA	58	956	15
	R=TCCTTAGAGTGCCACCCG	58		
<i>Acinetobacter</i>	sp4F CACGCCGTAAGAGTGCAATTA	58	294	16
	sp4R AACGGAGCTTGTCAGGGTTA	58		
<i>A. baumannii</i>	13TU,sp2FGTTCCTGATCCGAAATTCTCG	58	490	

nitrofrantoine (NIT) (300 µg). Results were interpreted according to the Clinical Laboratory Standards Institute (CLSI) guidelines¹².

Classification of MDR gram-negative Bacilli

MDR has been considered for clinically significant gram-negative Bacilli such as *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* based on the aforementioned antimicrobial resistance definition. Classes of antibiotics used for MDR-GNB analysis were aminoglycoside (AMG), cephalosporins (CEPH), carbapenems (CARB), and fluoroquinolones (FQ) as follows: bacteria that were MDR for four classes of antibiotics (AMG+ CEPH+ CARB+ FQ) and bacteria that were MDR for three classes of antibiotics (either AMG+CEPH +FQ, CARB+CEPH+FQ, AMG+CEPH+CARB, or AMG+FQ+CARB)³.

Cephalosporin resistance was defined as resistance to ceftriaxone and ceftazidime, except for *P. aeruginosa*, where only ceftazidime was used. Carbapenem resistance was defined as resistance to both meropenem and imipenem. Aminoglycoside resistance was defined as resistance to both gentamicin and amikacin. Ciprofloxacin resistance was considered an indication to fluoroquinolones resistance.

Ethical consideration

Formal permission was obtained from the managers of Soba University Hospital and the Institutional Research Ethics Committee of the Institute of Endemic Diseases, University of Khartoum, approved this study under reference number IEND_REC 12/2017. Patient consent was waived by the Research Ethics Committee.

Statistical analysis

Data were analysed using Microsoft Excel and SPSS version 20.0. Cross-tabulation was used to present the different relations between data, qualitative data were performed using a χ^2 test (significance was set at $p \leq 0.05$), which was performed to

find the differences between bacterial isolates with resistance to at least one class of antibiotics by specimens (blood, urine, wound and other samples) *p*-values were determined for primary and secondary outcomes.

Results

Bacterial identification

Isolated Gram-negative bacilli showed different strains, including *E. coli* (123 isolates, 17%), *K. pneumoniae* (249 isolates, 34%), *P. aeruginosa* (153 isolates, 21%), *A. baumannii* (75 isolates, 10%), *Burkholderia cepacia* (42 isolates, 6%), *Proteus mirabilis* and *Proteus vulgaris* (28 isolates, 4%), *Enterobacter coelestis* (28 isolates, 4%), *Stenotrophomonas maltophilia* (21 isolates, 2.8%) and other gram-negative bacilli (15 isolates, 2.2%).

While isolates were distributed among the different hospital units, most of the pathogenic strains were isolated from neonatal intensive care unit (ICU) (182 isolates, 24.8%) mainly *K. pneumoniae* (77 isolate, 42.3%) and pediatric units (175 isolates 23.8%) the most prevalent strains were *K. pneumoniae* (50 isolate, 29%) and *P. aeruginosa* (44 isolates, 25.1), while medicine (147 isolates, 20%), *K. pneumoniae* and *E. coli* (45 isolates 30.6%) for both. For the surgery unit (103 isolates, 14%) renal unit (78 isolates, 10.7%) and ICU (49 isolates, 6.7%) mainly *K. pneumoniae* (19 isolates, 39%), *P. aeruginosa* and *A. baumannii* were (9 isolates, 20.4) for both. *Klebsiella pneumoniae* was the most isolated organism from all hospital units. The distribution of different gram-negative isolates is shown in Table 2.

With regard to the distribution of the isolates among different clinical specimens, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were isolated mainly in blood specimens 39% and 25% respectively, while *K. pneumoniae* and *E. coli* were 36% and 30% of urine samples. *A. baumannii* was isolated from 14% of wound specimens and *P. aeruginosa* was isolated from 23%

Table 2. The incidence of gram-negative Bacilli isolated from patients admitted to different hospital wards in Khartoum State between October 2016 and February 2017.

Bacteria	Hospital ward						P-value
	ICU, n (%)	NICU, n (%)	Medicine, n (%)	Surgery, n (%)	Renal unit, n (%)	Paediatric, n (%)	
<i>Klebsiella pneumoniae</i> (249)	19 (39)	77 (42.3)	45 (30.6)	34 (33)	24 (31)	50 (29)	<0.001
<i>Escherichia coli</i> (123)	4 (8.1)	9 (5)	45 (30.6)	22 (21.3)	16 (21)	25 (14.2)	<0.001
<i>Pseudomonas aeruginosa</i> (153)	10 (20.4)	40 (21.9)	24 (16.3)	17 (16.5)	19 (24.3)	44 (25.1)	<0.001
<i>Acinetobacter baumannii</i> (75)	10 (20.4)	18 (9.9)	9 (6.1)	8 (8)	7 (9)	24 (14)	<0.001
<i>Burkholderia cepacia</i> (42)	3 (6.1)	11 (6)	7 (4.7)	6 (6)	4 (5.1)	11 (6.3)	<0.001
<i>Proteus</i> spp. (28)	1 (2)	3 (2)	4 (2.7)	9 (9)	3 (4)	8 (5)	<0.001
<i>Enterobacter</i> spp. (28)	1 (2)	10 (5.5)	4 (3)	3 (3)	4 (5.1)	6 (3.4)	<0.001
<i>Stenotrophomonas</i> spp. (21)	1 (2)	10 (5.5)	3 (2)	2 (2)	1 (1)	4 (2)	<0.001
Other gram-negative Bacilli (15)	0 (0)	4 (2.2)	6 (4)	2 (2)	0 (0)	3 (2)	<0.001
Total (734)	49 (6.7)	182 (24.8)	147 (20)	103 (14)	78 (10.7)	175 (23.8)	<0.001

*Other Gram-negative bacilli include *Citrobacter* species, *Serratia* species, *Vibrio vulnificus* and *Morganella morganii*. †Body fluids include cerebrospinal fluid, peritoneal fluid, pleural fluid, ascitic fluid and synovial fluid.

of wound specimens. For more details about gram-negative bacilli among different specimen see Table 3.

Antimicrobial resistance pattern of clinical isolates

Antibiotic resistance pattern are shown in Figure 1. Out of 734 isolates tested using the disk diffusion method, the highest percentage of resistance, in 97% and 93.5% of isolates, were found against ampicillin and cephalexin, respectively, followed by amoxicillin/clavulanic acid (90%), cefotaxime (89.7%), ceftriaxone (88.4%) and ceftazidime (79.2%). In addition, co-trimoxazole and nitrofurantoin resistance were detected in 74.4% and 75.2% of isolates, respectively. Resistance rates also were high in ciprofloxacin (45.2%), gentamicin (52.5%) and amikacin (18.3%). Meropenem and imipenem were the most effective antibiotic tested, with resistance observed in 21.6% and 16.2% of isolates, respectively.

The antimicrobial resistance patterns of most commonly isolated organisms are shown in Figure 2. *K. pneumoniae* resistant pattern as the following (22%) of them were resistant to meropenem while (11%) were resistant to imipenem, ceftazidime (80.6%), Gentamicin (52%), ciprofloxacin (42%) and amikacin (16.7%). With regard to the *E. coli* antimicrobial resistant pattern, meropenem (9%), imipenem (8%), ceftazidime (84.2%), ciprofloxacin (66.4%), Gentamicin (53.1%) and amikacin (12.0%). In *Pseudomonas aeruginosa* the resistance rate was meropenem (20%), imipenem (22%) ceftazidime (81%) followed by gentamicin (57.5%), ciprofloxacin (22.5%) and amikacin (9.5%). The rate of antimicrobial resistant among *A. baumannii* was as the following; meropenem (73.7%), imipenem (66.7%), amikacin (63.2%), gentamicin (79%) and ciprofloxacin (79%).

Multidrug resistance patterns among gram negative Bacilli

The gram-negative bacilli that were resistant to several antibiotic groups are shown in Table 4. Of 600 GNB isolates, 134

(22.3%) isolates were MDR. Of those MDR organisms, 48 isolates (8%) were resistant to four classes of antimicrobial drugs: *A. baumannii* (38 isolates, 50.6%), *K. pneumoniae* (9 isolates, 3.6%), and *E. coli* (1 isolate, 0.8%). A further 86 isolates (14.2%) were resistant to three classes of antimicrobial drugs: *A. baumannii* (38 isolates, 50.6%), *K. pneumoniae* (47 isolates, 18.8%), *P. aeruginosa* (21 isolates, 13.7%) and *E. coli* (6 isolate, 4.8%).

Discussion

Infection with MDR gram-negative Bacilli is a major problem worldwide, associated with increased patients morbidity and mortality¹⁷. In Sudan, the increasing number of MDR bacteria is a real clinical challenge^{18,19}. This study was undertaken; to identify the different patterns of AMR in bacterial pathogens isolated from patients treated in various wards of hospitals.

In this study, *K. pneumoniae* and *P. aeruginosa* strains were more prevalent in blood specimens while *K. pneumoniae* and *E. coli* strains were more frequently isolated in urine specimens.

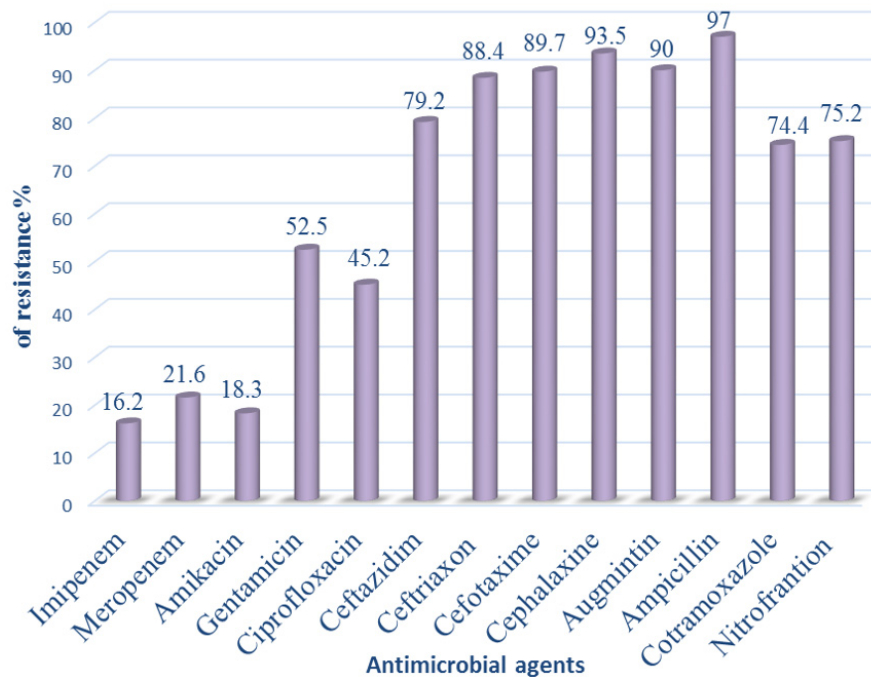
K. pneumoniae is a prominent pathogen causing both hospital-acquired and community-associated infections, including bacteremia, wound infection, pneumonia, urinary tract infections and other infections^{20,21}. In this study, *K. pneumoniae* was the most commonly isolated organism from the blood specimens mainly in neonatal sepsis in a high rate (39.8%). Most of these strains resistant to cephalosporins and other class of antibiotics including carbapenem as reported worldwide^{7,21,22}.

E. coli is regarded as the commonest pathogen of the urinary tract, causing complicated and uncomplicated urinary tract infections²³. In this study, the most frequently observed pathogens in urine specimens were *E. coli* (30%) and *K. pneumoniae* (36%). This finding is in harmony with the results of several other studies, including: de Francesco *et al.*, who found that *E. coli*

Table 3. The frequency of gram-negative bacilli isolated from different clinical samples obtained from patients treated at Khartoum State hospitals between October 2016 and February 2017.

Bacteria	Specimen						P-value
	Blood, n (%)	Urine, n (%)	Wounds, n (%)	Sputum, n (%)	Catheter tips, n (%)	Bodily fluids†, n (%)	
<i>Klebsiella pneumoniae</i> (249)	94 (39)	82 (36)	50 (27)	7 (32)	7 (28)	9 (29)	<0.001
<i>Escherichia coli</i> (123)	15 (6.1)	70 (30)	31 (17)	2 (9)	1 (4)	4 (12.9)	<0.001
<i>Pseudomonas aeruginosa</i> (153)	60 (25)	32 (14)	42 (23)	6 (27.2)	7 (28)	6 (25)	<0.001
<i>Acinetobacter baumannii</i> (75)	18 (7.4)	13 (5.6)	25 (14)	6 (27.2)	7 (28)	6 (25)	<0.001
<i>Burkholderia cepacia</i> (42)	20 (8)	9 (4)	9 (5)	1 (5)	1 (4)	2 (6.4)	<0.001
<i>Proteus</i> spp. (28)	6 (2.5)	7 (3)	12 (6.5)	0 (0)	2 (8)	1 (3.2)	<0.001
<i>Enterobacter</i> spp. (28)	13 (5)	8 (3.5)	7 (4)	0 (0)	0 (0)	0 (0)	<0.001
<i>Stenotrophomonas</i> spp. (21)	12 (5)	2 (0.9)	5 (3)	0 (0)	0 (0)	2 (6.4)	<0.001
Other gram-negative Bacilli* (15)	5 (2)	7 (3)	2 (1)	0 (0)	1 (0)	0 (0)	<0.001
Total (734)	243 (33.1)	230 (31.3)	183 (25)	22 (3)	26 (3.4)	30 (4.2)	<0.001

*Other Gram-negative bacilli include *Citrobacter* species, *Serratia* species, *Vibrio vulnificus* and *Morganella morganii*.

**Figure 1.** Antimicrobial resistance pattern among different gram-negative Bacilli isolated from patients treated at Khartoum State hospitals between October 2016 and February 2017.

was the commonest GNB (42.4%) isolate from urine specimens of patients with urinary tract infection²⁴; a study in Tanzania reported 38% of *E.coli* isolated from urinary specimens²⁵ and other studies from Pakistan and India^{25,26}.

In this study, non-lactose-fermenting gram-negative bacilli such as *P. aeruginosa* and *A. baumannii* were mostly encountered

in ICU patients, these organisms were isolated in 20.4% of samples, and were found in different clinical samples such as: blood, wound and sputum. This finding agrees with a study by Vincent *et al.* that reported *P. aeruginosa* and *A. baumannii* were frequently isolated from ICU patients by Vincent *et al.*²⁷; Jitendra *et al.*, reported that *A. baumannii* was the second most common pathogen in an ICU of a tertiary care center²⁸.

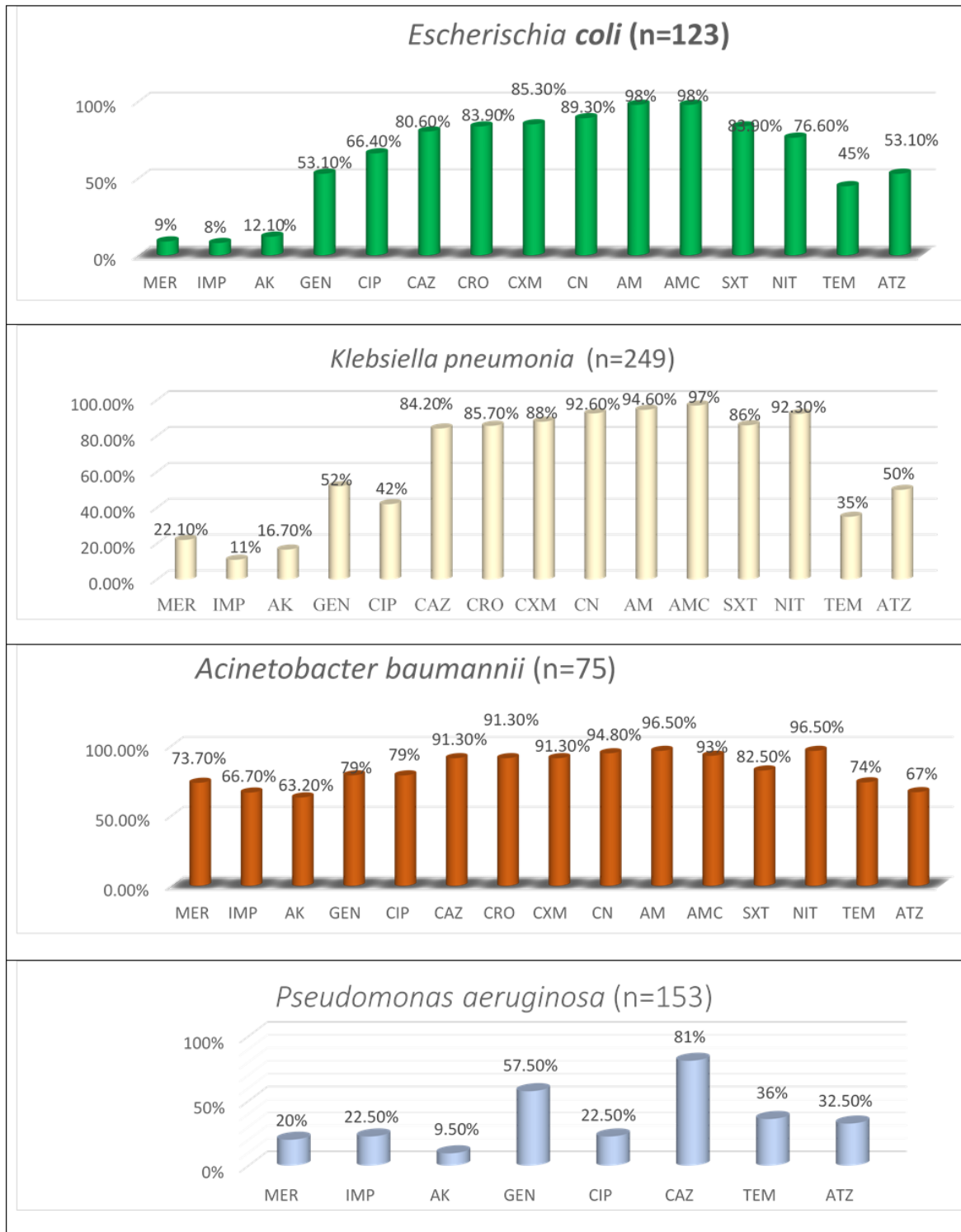


Figure 2. Antimicrobial resistance pattern of commonly isolated organisms' different antibiotics between October 2016 and February 2017. MER, meropenem; IMP, imipenem; AK, amikacin; GEN, gentamicin; CIP, ciprofloxacin; CAZ, ceftazidime; CRO, ceftriaxone; CXM, cefuroxime; CN, cephalixin; AM, ampicillin; AMC, amoxycillin-clavulanate; SXT, trimethoprim-sulfamethoxazole; NIT, nitrofurantoin; TEM, temocillin; ATZ, azetronam.

Table 4. Multidrug resistance among gram-negative isolates.

Class	Resistance to	<i>Klebsiella pneumoniae</i> , n (%)	<i>Escherichia coli</i> , n (%)	<i>Pseudomonas aeruginosa</i> , n (%)	<i>Acinetobacter baumannii</i> , n (%)	Total, n (%)
All isolates	NA	249	123	153	75	600
4MDR	AMG+ CEPH+ FQ+ CARB	9 (3.6%)	1 (0.8%)	0 (0%)	38(50.6%)	48 (8%)
3MDR	AMG+ CEPH+ FQ	31(12.4%)	5(4%)	4 (2.6%)	2 (2.6%)	42 (7%)
3MDR	CARB+ CEPH+ FQ	6 (2.4%)	0 (0%)	8 (5.2%)	8 (10.6%)	22(3.6%)
3MDR	AMG+ CEPH+ CARB	2 (0.8%)	0 (0%)	5 (3.2%)	1 (1.3%)	8 (1.3%)
3MDR	AMG+ FQ+ CARB	8 (3.2%)	1(0.8%)	4 (2.6%)	1 (1.3%)	22(3.6%)
Total		56 (22.4%)	7 (5.6%)	21 (13.7%)	50 (66.6%)	14 (2.3%)

NA, not applicable; AMG, aminoglycoside; CEPH, cephalosporins; FQ, florquinolones; CARB, carbapenem.

Concerning the infection type, we found that *P. aeruginosa* were associated with 25% of blood stream infections and 23% of wound infection, while *A. baumannii* mainly associated with wound infection in 14% in agree with Gales *et al.* 2010²⁹.

Resistance of gram-negative bacilli is widespread and multi-drug resistance has been reported in many studies^{2,4,5}, causing challenges in the treatment of nosocomial infections. The resistance pattern was commonly reported in classes such as cephalosporins, carbapenem, aminoglycosides and quinolones^{30–32}. In this study, we observed high rates of resistance to extended-spectrum β -lactamases (ESBL), resistance to cephalixin, cefuroxime, ceftazidime and ceftriaxone, in addition to resistance to ampicillin and amoxicillin/clavulanic acid.

The analysis of the antimicrobial susceptibility patterns of the study isolates showed high rate of MDR organisms that were resistant to three or more classes of antibiotics, including carbapenem and aminoglycosides. This pattern was observed mainly among *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

The most resistant strain was *Acinetobacter baumannii*, being resistant to all four classes of antibiotics used in 50.6% of isolates. A total of 73.7% of *A. baumannii* were found to be resistant to meropenem, and 66.7% to imipenem, while in the cephalosporin class, more than 91% of the isolates were resistant. *A. baumannii* also have high resistance rate to aminoglycosides and quinolones (63.2% for amikacin and 79% for both gentamicin and ciprofloxacin). This increasing resistance among *A. baumannii* has become a public-health issue, because this bacterium frequently causes nosocomial infections³¹.

In this study the most common clinical isolates of the Enterobacteriaceae family were *K. pneumonia* and *E. coli*. In both species, there was a high prevalence of resistance against quinolones, aminoglycosides and beta-lactams. Rates of resistance to carbapenems were alarmingly high: 22% in *K. pneumoniae* and 9% in *E. coli*. These bacteria have high resistance rate to ceftazidime

and cephalixin, (80.6% and 92%, respectively). These rates are much higher than those observed in 2013 by Ali in Soba University hospital, who found that ceftriaxone and ceftazidime resistance rates ranged from 56% to 79%^{21,33}. Aminoglycoside (specifically amikacin) resistance rates among *K. pneumoniae* and *E. coli* were 16.7% and 12.1%, respectively, which is higher than those observed by Lee in 2013, who found the resistance rate to amikacin was 6.2% in *K. pneumoniae* and 1.3% in *E. coli*³⁰. In this study Gentamicin resistance among both *K. pneumoniae* and *E. coli* was 53%, which is a high resistance rate. *E. coli* was highly resistant to quinolones like ciprofloxacin (in 66.4%), whereas *K. pneumoniae* was resistant in 42% of isolates. This finding is much lower than that observed by Moolchandani *et al.* in 2017³².

Pseudomonas aeruginosa was resistant to carbapenem in 22% of isolates and was highly resistant to ceftazidime (in 81% of isolates) followed by gentamicin (57.5%), ciprofloxacin (22.5%) and amikacin (9.5%). resistance to many antibiotic among *P. aeruginosa* was reported in many studies^{4,34}.

The high level of resistance in the current study can be attributed to the unrestricted use of antibiotics in Sudanese hospitals; this injudicious use has been shown to have, an important role in increasing carbapenem resistance³². During this study, 134 gram negative bacilli resistant to three or four classes of antibiotics were isolated over a period of six months, which is relatively higher rate than the rate reported in a previous study also conducted in SUH over 30 months, from January 2011 to June 2013³³. This concerning finding indicates the rapid acceleration in the rate of emergence of MDR organisms in our local sittings. Moreover, 80 bacterial strains were resistant to all available antibiotics, including meropenem.

In addition, microbiology laboratories play a crucial role against the spread of antimicrobial resistance by accurately identifying causative pathogens and detecting their antimicrobial susceptibility profile, so as to guide the proper use of antibiotics by the care providers. Unfortunately, in Sudan the lack of reliable

microbiology laboratory services adds yet another layer to the multifactorial problem of MDR organisms' epidemic in our country. Most of the laboratories in our country use the disk diffusion method for antimicrobial susceptibility testing; however, there are no policies regarding phenotypic screening for antibiotic resistance among certain organisms; resulting in that the laboratory report may not be accurate and misguide the doctors. For instance, in the disk diffusion method, if the inhibitory zone around ceftazidime was ≤ 22 mm, ideally this should trigger testing for the presence of ESBLs in the isolated pathogen, because the presence of these enzymes renders all penicillins, cephalosporins and monobactams¹² useless against that certain isolate, even if they shows susceptible results in the routine test. Yet, this extra step is not taken and only the routine susceptibility results will be reported, which may inaccurately show some antibiotics as susceptible while in reality the organism is resistant to them. Lastly, there is a limited choice of available antibiotics in Sudan; the most widely available are cephalosporins, which makes them the backbone of treating infectious diseases regardless of the isolate or its susceptibility profile. Carbapenems are believed to treat all evils, once they were reserved for the treatment of MDR organisms causing systemic infections⁴, but the recent trend led to over use them for treating infections that can be managed by less potent antibiotics, contributing to this vicious cycle that aggravate the AMR issue even more.

Conclusion

In conclusion, there was a high prevalence of gram negative bacterial pathogen associated hospital and community acquired

infections, with increasing rates of resistance to available antibiotics. Strict infection control measures should be implemented, and antimicrobial stewardship should be initiated and policed to decrease the spread of MDR pathogens in Sudanese hospitals.

Data availability

Raw data for the present study, including the genotypes, isolation location and resistance status of each bacterial isolate, is available of figshare. DOI: <https://doi.org/10.6084/m9.figshare.7584449>³⁵.

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

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