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Periodic phenotypic profile analysis of *Acinetobacter baumannii* drug resistance characteristics showing the emergence of PDR

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Abstract. – OBJECTIVE: ***Acinetobacter baumannii*, one of the ESKAPE pathogens, is on the World Health Organization (WHO) list of priorities needing urgent new effective antimicrobial agents due to exhibited high resistance by the bacterium to currently available antibiotics. This study examines the periodic changes of clinical *A. baumannii* isolates and their antimicrobial resistance patterns and types in Southeastern region of Saudi Arabia.**

MATERIALS AND METHODS: One hundred and seventy-seven randomly selected *A. baumannii* isolates were used for the investigation with bacterial identities (IDs) and antimicrobial assay ascertained with Gram-negative (GN) ID cards and antimicrobial susceptibility test (AST) cards of Vitek 2 Compact Automated System according to manufacturer's guidelines. Descriptive phenotypic types of isolates were compared using comparison proportion and Fisher extraction test, while Morpheus versatile matrix visualization and analysis software was used for the dendrogram of hierarchical clustering.

RESULTS: A significantly higher proportion of samples were from males compared to females ($p = 0.025$), with 33.33% of samples originating from patients aged 51-70 years. Resistance was high for imipenem (93%), meropenem (94%), levofloxacin, ciprofloxacin (99%), and aztreonam (98%). There was less percentage resistance to colistin (18%), tigecycline (23%), and minocycline (23%). Multidrug resistance (MDR)/ carbapenem-resistant *Acinetobacter baumannii* (CRAB) was observed consistently across all years. There was no extensive drug resistance (XDR) among isolates from 2013 to 2014, but it was present in the 2016 to 2018 and 2019 to 2020 periods, while pandrug resistance was seen only in the 2019 to 2020 isolates.

CONCLUSIONS: The study shows a clear trend of the isolates changing from MDR to XDR and then to pandrug resistance over the study period. Also, it indicates that carbapenems might no longer be a treatment choice in this study region. Although colistin exhibited less resistance, the toxicity of the drug reduces its usefulness. The development of pandrug resistance is a critical concern.

Key Words:
Acinetobacter baumannii, Antimicrobial resistance, Multidrug resistance, Extensive drug resistance, Pandrug resistance, Carbapenem resistance.

Introduction

In a recent CLSI news report[1], *Acinetobacter baumannii* was described as "the bad, the awful, and the downright ugly", a characterization that aptly reflects the current status and concerning traits of this opportunistic pathogen. With an estimated 8,500 carbapenem-resistant cases of infection amongst critically ill patients in the USA[2], mortality rates in severe cases are placed at between 14-73% in the US[3,4], with percentages differing in various regions of the world[5]. A recent study[6] indicates that the mortality rate ranges from 18% to 88%, depending on the source of the infection and varying geographically. Overall, *A. baumannii* is recognized as one of the leading causes of nosocomial infections, associated with high morbidity and mortality[7]. Consequently, in the 21st century, this bacterium has emerged as a significant global public health threat, linked to both community-acquired and hospital-associated infections, constituting a great challenge to clinicians. Besides, this opportunistic pathogen has evolved over time and is presently grouped as one of the ESKAPE pathogens[8,9].

In addition to this, *Acinetobacter baumannii* has emerged as the most important species of the genus *Acinetobacter*[5]. The bacterium is an op-

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portunistic pathogen commonly associated with hospital-acquired infections (HAIs) and highly resistant to available antimicrobials[10]. Furthermore, it is postulated to be the third most common bacteria pathogen causing bacteremia in patients and the seventh most common isolated bacterium recovered from critically ill patients-[11]The bacterium is also recognized as one of the six most significant antibiotic-resistant pathogens associated with hospitalized patients worldwide, exhibiting an exceptional ability to spread across various environments[12]. An estimated 10 million deaths could be attributed to *A. baumannii* by 2050 if the current trend of antimicrobial resistance (AMR) continues unchecked[13,14].

Carbapenems were once the preferred treatment for *A. baumannii* infections, but their frequent use has led to a significant rise in resistance to these antibiotics[15]. This resistance has now become widespread[16] with an increase in carbapenem-resistant strains has been reported in Saudi Arabia[17,18]. This has subsequently resulted in reduced therapeutic efficacies of these drugs as well as providing clinicians with fewer options in the management of hospitalized patients with *A. baumannii* infections. The resistance of *Acinetobacter baumannii* to antimicrobials has diverse and complex mechanisms that contribute to treatment complications[19]. Generally, carbapenem-resistant *Acinetobacter baumannii* (CRAB) has been an increasing major health challenge associated with high mortality among intensive care unit (ICU) patients[20].

Over the years, CRAB has advanced from multidrug resistance (MDR), with reports[21,22] from the United Kingdom showing an increase in resistance from 47% to 77% in just three years. Elsayed et al[23] also documented the presence of not only MDR but extensive drug resistance *A. baumannii* (XDR-AB) with the presence of pandrug resistance *A. baumannii* among patients. Therefore, the progression from MDR to pandrug resistance is concerning, especially with the increasing difficulty in treating bacterial infections.

For all the aforementioned reasons, *A. baumannii* is listed by the World Health Organization[24,25] as a critical pathogen of priority needing the development of effective drugs as a result of high resistance to currently available antibiotics[26].

MDR is defined by the Centers for Disease Control and Prevention (CDC)[27] as resistance to at least one agent in three or more classes of antibiotics, including the aminoglycosides, cephalosporins, carbapenems, fluoroquinolones, piperacillin-tazobactam, ampicillin-sulbactam. Therefore, MDR has now constituted a clinical challenge leading to high morbidity and mortality in critically ill patients. Also, XDR is the nonsusceptibility by an isolate to a minimum of one agent in all but two or fewer classes of antibiotics. Pandrug-resistant (PDR) *A. baumannii* are categorized as those that are extensively drug-resistant (XDR) and additionally resistant to tigecycline and polymyxins[28]. MDR, XDR, and PDR *A. baumannii* clinical isolates are on the rise creating difficulties in defining standard treatment for patients. MDR *A. baumannii* (MDR-AB) is reported to be on the increase globally[29,30]. Generally, XDR and PDR *A. baumannii* have been reported in regions of the world and are gaining much attention by researchers[31,32]. Therefore, clinicians are challenged with the few therapeutic options available for managing critically ill patients. According to studies[33], frequently adduced mechanisms of *A. baumannii* resistance include beta-lactamase expression, the presence of porins, and efflux pumps. Therefore, resistance acquisition of the bacterium has been well documented in the literature, with their resistance to quinolones reported to be due to mutations in the gyrA and parC genes, whereas, for aminoglycosides, it expresses the presence of modifying enzyme[34]. Also, its resistance to colistin is due to mutation in PmrA and B proteins[35]. These agents are drugs used in the control of Gram-negative bacterial infections. Therefore, in cases of *A. baumannii* infection management, it is important to determine the likelihood of resistance due to prior antibiotic exposure, previous Gram-negative culture for MDR-AB, and environmental rates of resistance. Despite the aforementioned, clinical practice has been in favor of using polymyxin-carbapenem as combined therapeutic measures even without any evidence of better outcome[36]. This might, therefore, elucidate the reason why there has been a progression of this ubiquitous pathogen from MDR strains to emerging pandrug-resistant ones, further complicating the management of resultant infections.

Extreme drug-resistant *A. baumannii* (XDR-AB) is reportedly a "dreaded entity" in nosocomial bacteremia and ventilator-associated pneumonia infections in hospitalized patients[37]. To portray concerns for difficult-to-treat *A. baumannii*, it has also been designated as a "red alert", creating difficulties for clinicians due to the spectrum of XDR[38]. The WHO's[39] warning about

a post-antibiotic era, where common infections could become fatal, still stands with no relief in sight. Much literature is available on the mechanism of antimicrobial resistance and its molecular mechanism in *A. baumannii*. There will be a need to re-examine the phenotypic characteristics of the bacterial strains in this region of study for surveillance and curtailment. Therefore, the present investigation seeks to look into the phenotypic characteristics of clinical isolates of *A. baumannii* over the periods of 2013-2014, 2016-2018, and 2019-2020. In addition, it examines the trends in changes in susceptibility in terms of their antimicrobial resistance patterns. Finally, to ascertain the reason for the rise in treatment failure, particularly MDR, XDR, and pan drug-resistant clinical isolates of *Acinetobacter baumannii* from this Southeastern region of Saudi Arabia. A region that had reported a high incidence of carbapenem-resistant *A. baumannii* [CRAB] strains in a number of research[17,18,40]. The goal is to update antimicrobial susceptibility patterns over time, in accordance with regional surveillance recommendations.

Materials and Methods

A. baumannii isolates were stored in the microbank at -80°C freezer in the laboratory of the Microbiology division of the College of Medicine, King Faisal University, Al-Ahsa. No patients were involved in the study.

Bacterial Isolates and ID Confirmation

Randomly selected *Acinetobacter baumannii* isolates were retrieved from Microbank™ tubes (Pro-lab Diagnostic, Georgetown, TX, USA) that had been stored in a -80°C freezer. Each isolate had codes that differed depending on the year of the first isolation. They were retrieved by plaiting them out on MacConkey agar and incubated overnight at 37°C. Pure bacterial colonies were obtained by plaiting out the resultant overnight growth on MacConkey agar, incubated at 37°C for 24 hours, and used for confirmation of IDs and antimicrobial susceptibility test of the bacterial isolates. The bacterial isolate IDs were confirmed using the Gram-negative (GN) ID cards of Vitek 2 Compact Automated System (BioMérieux, Marcy L'Etoile, France) following the manufacturer's guidelines. Also, the detection of the *OXA-51* gene by PCR using previously described primers sequence, *F: 5'-TAATGCTTTGATCGGCCTTG-3', R: 5'-TGGATTGCACTTCATCTTGG-3* confirmed the *A. baumannii* isolates[18,41].

Antimicrobial Susceptibility Test (AST), Minimum Inhibitory Concentration, and ESBL assay

With the GN AST cards of Vitek 2 Compact Automated System (BioMérieux, Marcy L'Etoile, France), the isolates were tested against the following antibiotics: Benzylpenicillin (BENPEN), Ampicillin/Sulbactam (Ams), Ceftazidime (Caz), Cefepime (Pime), Ticarcillin/Clavulanic Acid (Tcc), Imipenem (Imp), Meropenem (Mer), Tigecycline (Tig), Colistin (Cs), Ciprofloxacin (Cip), Gentamicin (Gm), Amikacin (Amk), Tobramycin (Tob), Netilmicin (Net), Minocycline (Min), Aztreonam (Azt), Levofloxacin (Levo), Ampicillin (Amp), Trimethoprim/sulfamethoxazole (Sxt), Piperacillin/Tazobactam (Ptz), according to the guidelines of the manufacturers (https://www.epa.gov/sites/default/files/2017-01/documents/qc-22-04.pdf, accessed on 31/01/2024). The minimum inhibitory concentrations for the antibiotics and the Extended Spectrum Beta-Lactamase (ESBL) production were defined with Vitek 2 Compact Automated System (BioMérieux, Marcy L'Etoile, France). Results from the antimicrobial assay were used to define the isolates into MDR, XDR, and PDR as defined by the CDC and the European Center for Disease Prevention and Control (ECDC)[41-44].

Grouping of Antibiotics

The antimicrobials were grouped into the following groups: Polymyxins (colistin), Penicillins (Benzylpenicillin, Ampicillin), β-lactamase inhibitors (Ampicillin/Sulbactam), Antipseudomonal penicillin's plus, b-lactamase inhibitors (Piperacillin/Tazobactam), Fluoroquinolones (Levofloxacin, ciprofloxacin), Extended-spectrum cephalosporins (Ceftazidime, Cefepime), Carbapenem (Imipenem, Meropenem), Monobactam (Aztreonam), Tetracyclines (Minocycline, Tigecycline), and Folate pathway inhibitors (Trimethoprim/sulfamethoxazole).

Statistical Analysis

An Excel sheet was used for the collection of data, with results presented as numbers and percentages. The descriptive types of *A. baumannii* resistance profiles were compared statistically using comparison proportion and Fisher's extraction test. Also, GraphPad Prism version

10.0.2 (232) (Boston, MA, USA) was used to compute the heatmap display. The correlation between percentage resistance *vs.* percentage sensitivity and intermediate resistance was computed using a two-tailed Pearson correlation coefficient test at a 95% confidence interval and significance taken at $p \leq 0.05$. Also, data comparing the percentage of resistance according to antimicrobial groups are presented as mean ± SD. While dendrogram of hierarchical clustering was generated with Morpheus versatile matrix visualization and analysis software (https://software.broadinstitute.org/morpheus).

Results

Demographics and Sample Types

The patient demographics and types of samples from which *A. baumannii* was isolated are presented in **Supplementary Table I**.

There were significantly more samples from males (59.88%) than females (38.42%) (p = 0.025), with sex not specified (NS) for 1.7% of the samples. The patients' ages ranged from 12 months to 90 years.

The majority of them were in the age groups of 61-70 years (17.51%) and 51-60 years (15.82%), with differences not significant (p = 0.749). In addition to this, most of the isolates were from transtracheal aspirate (35.02%) and wound swabs (30%), with differences in percentage that were not statistically significant (p = 0.449). Also, high were isolates from Sputum (12.3%), while other specimen types included blood (2.82%), bronchial alveolar (1.69%), central venous catheter (CVC) (1.69%), cerebral spinal fluid (CSF) (1.23%), nasopharyngeal aspirates (1.69%), urine (3.4%), tissue culture (1.69%) and incision drainage fluid (3.95%). In 4.52% of specimen types were not specified (NS) (**Supplementary Table I**).

Description of A. Baumannii Isolates and Susceptibility Profile

A total of 177 isolates collected between 2013 and 2020 were randomly selected for the investigation. Of these, 38 (21.47%) were from the 2013-2014 samples, with their characteristics displayed in Table I. The results in Table I show varying percentages of susceptibility among the 2013-2014 isolates, with susceptibility to one antibiotic (13%), two antibiotics (26.3%), three antibiotics (21%), and up to four or five antimicrobials (18.42% and 15.8%, respectively). Only two (5%) of the *A. baumannii* isolates in this group were classified as susceptible strains; however, the majority of the isolates were significantly (p = 0.000002) identified as MDR/CRAB strains (71%), while the rest were categorized as either XDR/CRAB (13%) or MDR carbapenem-susceptible strains, as detailed in Table I. The isolates here were sensitive to colistin (Cs), which is the only antimicrobial to which the XDR/CRAB isolates were sensitive. There was also a high susceptibility to Tig, as seen in 28 (73.68%) of the 2013-2014 isolates.

The majority (62.71%) are the 2016-2018 isolates, and their profiles are described in Tables II-IV, grouped according to the number of antimicrobials to which they were susceptible. To this effect, the results describing the profile of isolates sensitive to one or two antimicrobials are displayed in Table II, while those susceptible to three, four, or more antibiotics are shown in Tables III and IV, respectively.

A description of 38 isolates collected between 2016-2018 that were sensitive to one or two antimicrobials is shown in Table II. The isolates were either significantly (p = 0.001) more (97%) MDR/CRAB or XDR (3%). All the isolates in this group were resistant to the carbapenems, mostly sensitive to Cs (82%) and Tig (59%). Another twenty-nine of the 2016-2018 *A. baumannii* isolates were sensitive to only three antibiotics, and their profile description is shown in Table III. The table shows that all the isolates were MDR/CRAB, and a high number of them (96%) were sensitive to colistin (Cs) and Tig (89.65%), while sensitivity to other antibiotics varied (Table III).

The susceptibility profile and description of the remaining forty-eight isolates of the 2016-2018 group are shown in Table IV. These isolates in this table were sensitive to four or more antibiotics, and all the isolates, with the exception of one (09 A156), were sensitive to both Tig and Cs. The results also (Table IV) showed different *A. baumannii* isolates with the classification of resistant patterns along with the number of antimicrobials they are sensitive to. Only five isolates were found to be sensitive to the antibiotics tested. These sensitive results, when compared to other resistant patterns, appeared to be significantly low ($p < 0.0001$), representing 10.42%. This indicates that many of the *A. baumannii* isolates are highly resistant strains. Four *A. baumannii* isolates (8.33%) exhibited MDR characteristics.

Table I. Description of *A. baumannii* isolates collected between 2013 and 2014.

Bacterial isolate code	Susceptibility pattern	Susceptible antibiotics	Number sensitive	Percentage (%) sensitive	Total
08 A3	MDR/CRAB	Tig, Cs, Ptz	3	18.7	
08 A19	MDR/CRAB	Tig, Cs, Net	3	18.7	
08 A23	MDR	Cs, Imp, Mer, Amk	4	25.0	
08 A27	MDR	Mer, Tig, Cs	3	15.8	
08 A30	XDR/CRAB	Cs	1	6.7	
08 A33	MDR/CRAB	Cs, Amk	2	13.3	
08 A36	MDR/CRAB	Cs, Tig, Sxt	3	15.8	
08 A42	MDR/CRAB	Tig, Cs	2	14.3	
08 A43	MDR/CRAB	Tig, Cs	2	14.3	
08 A67	XDR/CRAB	Cs	1	6.7	
08 A68	MDR/CRAB	Tig, Cs, Tob, Min	4	22.2	
08 A74	MDR/CRAB	Tig, Cs	2	13.3	
08 A75	MDR/CRAB	Tig, Cs, Amk	3	21.4	Total = 38
08 A76	MDR/CRAB	Tig, Cs, Amk, Tob, Net, Min	6	33.3	MDR = 4 (11%)
08 A88	MDR/CRAB	Tig, Cs, Sxt	3	15.8	MDR/CRAB = 27 (71%)
08 A89	MDR/CRAB	Tig, Cs, Sxt	3	15.8	XDR/CRAB = 5 (13%)
08 A92	MDR/CRAB	Tig, Cs	2	13.3	SS = 2 (5%)
08 A93	XDR/CRAB	Cs	1	6.7	
08 A94	XDR/CRAB	Cs	1	6.3	
08 A98	MDR	Imp, Tig, Cs, Net	4	28.6	Fisher's exact test =
08 A112	MDR/CRAB	Tig, Cs	2	14.3	Significance level
08 A113	MDR/CRAB	Tig, Cs	2	14.3	
08 A114	MDR/CRAB	Tig, Cs	2	14.3	$p = 0.000002$
08 A119	MDR/CRAB	Tig, Cs	2	14.3	
08 A122	MDR/CRAB	Tig, Cs	2	14.3	
08 A132	MDR	Imp, Mer, Tig, Cs, Net	5	27.8	
08 A159	MDR/CRAB	Tig, Cs, tz	3	18.7	
08 A160	XDR/CRAB	Cs	1	7.1	
11 A4	SS	Imp, Mer, Tig, Cs, Tob, Net, Min	7	41.2	
11 A9	SS	Tig, Cs, Amk, Tob, Net, Min, Ptz	7	38.8	
11 A13	MDR/CRAB	Cs, Ams, Tob, Net	4	23.5	
11 A14	MDR/CRAB	Tig, Cs, Tob, Net	4	23.5	
11 A44	MDR/CRAB	Tig, Cs, Tob, Net, Min	5	29.4	
11 A46	MDR/CRAB	Cs, Ams, Tob, Net	4	23.5	
11 A47	MDR/CRAB	Cs, Tob, Net,	3	17.6	
11 A158	MDR/CRAB	Tig, Cs, Tob, Net, Min	5	27.7	
11 A175	MDR/CRAB	Tig, Cs, Tob, Net	4	25.0	
10 A179	MDR/CRAB	Tig, Cs, Min	3	16.7	

MDR: multidrug-resistant; XDR: extensive drug-resistant; CRAB: carbapenem-resistant *Acinetobacter baumannii*; SS: susceptible strain; Imp: Imipenem; Mer: meropenem; Amk: amikacin; Tig: tigecycline; Cs: colistin; Tob: tobramycin; Net: netilmicin; Ptz: piperacillin/tazobactam; Ams: ampicillin/sulbactam; Min: minocycline.

Furthermore, the majority of the remaining isolates (81.25%) displayed dual MDR-CRAB traits, with a statistically significant difference ($p < 0.0001$) based on a comparison of proportions analysis. Table V describes the characteristics of the 2019-2020 *A. baumannii* isolates. The number of antibiotics against which the isolates were sensitive varied. Of the twenty-eight isolates in this group, 4 (11%) were not sensitive to any of the antibiotics, including Tig and Cs, and thus PDR. All the 2019-2020 isolates were carbapenem-resistant, while significantly ($p = 0.0158$) more of them (53%) were MDR/CRAB as compared to those that were XDR/CRAB (36%).

Overall Antimicrobial Susceptibility by the Isolates and MICs of Tested Antimicrobials

Figure 1A displays a summary of the findings on 177 *A. baumannii* isolates antimicrobial agents' susceptibility types in terms of sensitivity, intermediate, and resistance. Results showed the isolates were highly resistant to imipenem and meropenem (93% and 94%, respectively).

Table II. Description of 2016-2018 *A. baumannii* isolates susceptible to two or three antimicrobials.

Bacterial isolate code	Drug resistan pattern	Susceptible antibiotics	Number sensitive	Percentage (%) sensitive	Total
09 A20	XDR/CRAB	Ams	1	5.5	
09 A34	MDR/CRAB	Cs	1	6.3	
09 A72	MDR/CRAB	Cs	1	7.1	
09 A121	MDR/CRAB	Tig	1	6.3	
09 A137	MDR/CRAB	Cs	1	7.7	
09 A149	MDR/CRAB	Cs	1	6.7	
09 A166	MDR/CRAB	Cs	1	5.5	
09 A180	MDR/CRAB	Cs	1	6.3	
09 A181	MDR/CRAB	Tig	1	6.3	
09 A11	MDR/CRAB	Cs, Sxt	2	11.8	34 isolates
09 A71	MDR/CRAB	Cs, Sxt	2	11.8	XDR/CRAB = 1 (3%)
09 A24	MDR/CRAB	Tig, Cs	2	11.1	MDR/CRAB = 33 (97%)
09 A29	MDR/CRAB	Cs, Sxt	2	12.5	
09 A52	MDR/CRAB	Tig, Cs	2	11.1	
09 A53	MDR/CRAB	Tig, Cs	2	12.5	$p < 0.0001$
09 A61	MDR/CRAB	Tig, Cs	2	15.4	Comparison of proportion
09 A62	MDR/CRAB	Tig, Cs	2	15.4	comparison
09 A63	MDR/CRAB	Tig, Cs	2	13.3	
09 A80	MDR/CRAB	Tig, Cs	2	13.3	
09 A81	MDR/CRAB	Tig, Cs	2	14.3	
09 A82	MDR/CRAB	Tig, Cs	2	15.4	
09 A83	MDR/CRAB	Tig, Cs	2	11.1	
09 A85	MDR/CRAB	Tig, Cs	2	16.7	
09 A86	MDR/CRAB	Tig, Cs	2	15.4	
09 A130	MDR/CRAB	Tig, Cs	2	14.3	
09 A148	MDR/CRAB	Tig, Cs	2	13.3	
09 A152	MDR/CRAB	Tig, Cs	2	12.5	
09 A170	MDR/CRAB	Tig, Cs	2	12.5	
09 A154	MDR/CRAB	Ams, Tob	2	11.8	
09 A104	MDR/CRAB	Cs, Sxt	2	11.8	
09 A182	MDR/CRAB	Cs, Sxt	2	12.5	
09 A139	MDR/CRAB	Tob, Net	2	12.5	
09 A141	MDR/CRAB	Tig, Cs	2	11.8	
09 A146	MDR/CRAB	Tig, Cs	2	14.3	

MDR: multidrug-resistant; XDR: extensive drug-resistant; CRAB: carbapenem-resistant *Acinetobacter baumannii*; Tig: tigecycline; Cs: colistin; Tob: tobramycin; Net: netilmicin; Sxt: trimethoprim/sulfamethoxazole; Ams: ampicillin/sulbactam.

They were less resistant to amikacin, netilmicin, and tobramycin, with 79, 41, and 54%, respectively. Additionally, these isolates showed a higher resistance to ciprofloxacin and levofloxacin at 99% each as compared to all antimicrobial agents used in this study. However, resistance by the isolates was significantly lower to colistin (18%), tigecycline (23%), and minocycline (23%), thus more sensitive to these agents as compared to other antimicrobials. However, resistance to aztreonam was found to be 98%, whereas resistance to ampicillin-sulbactam and piperacillin-tazobactam showed 84% and 91%, respectively. Furthermore, *A. baumannii's* resistance to benzylpenicillin and ampicillin rightly exhibited 100% resistance. The bacterium is known to be intrinsically resistant to the two penicillin antibiotics. In terms of antimicrobial grouping (Figure 1B), penicillin, as expected, showed absolute resistance with antipseudomonal penicillin plus beta-lactam inhibitor, having 93.5%. *A. baumannii* isolates exhibited 98% and 99% resistance to monobactam and fluoroquinolones. Resistance to aminoglycosides and tetracyclines appeared to be low, but polymyxin had the lowest resistant profile, as exhibited by *A. baumannii* isolates.

The results of minimum inhibitory concentrations given by Vitek 2 Compact Automated System for the isolates are shown in **Supplementary Table II**. Results were interpreted by the 2015 Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Anti-

Table III. The characteristics of *A. baumannii* isolates from 2016 to 2018 are sensitive to three antibiotics.

Bacterial isolate code	Susceptibility pattern	Susceptible antibiotics	Number (%) sensitive	Total
09 A1	MDR CRAB	Tig, Cs, Tob	17.6	MDR = 29 CRAB = 29
09 A2		Tig, Cs, Tob	17.6	
09 A5		Tig, Cs, Tob	16.7	
09 A10		Tig, Cs, Amk	18.8	
09 A15		Tig, Cs, Net,	20.0	
09 A18		Tig, Cs, Min	16.7	
09 A25		Tig, Cs, Amk	20.0	
09 A32		Tig, Sxt, Ptz	18.8	
09 A39		Tig, Cs, Net,	17.6	
09 A45		Tig, Cs, Ams	16.7	
09 A54		Tig, Cs, Ptz	23.0	
08 A56		Tig, Cs, Levo	21.4	
09 A70		Tig, Cs, Ptz	20.0	
09 A99		Tig, Cs, Amk	21.4	
09 A111		Cs, Ams, Sxt	16.7	
09 A116		Tig, Cs, Sxt	16.7	
09 A126		Tig, Cs, Min	16.7	
09 A128		Tig, Cs, Min	16.7	
09 A129		Tig, Cs, Min	16.7	
09 A131		Tig, Cs, Tob	16.7	
09 A140		Tig, Cs, Min	16.7	
09 A144		Cs, Ams, Ptz	16.7	
09 A145		Tig, Cs, Amk	21.4	
09 A151		Tig, Cs, Sxt	18.8	
09 A153		Tig, Cs, Amk	18.8	
09 A171		Tig, Cs, Tob	16.7	
09 A172		Tig, Cs, Tob	16.7	
09 A167		Cs, Min, Azt,	16.7	
09 A168		Tig, Cs, Min	16.7	

MDR: multidrug-resistant; CRAB: carbapenem-resistant *Acinetobacter baumannii*; Amk: amikacin; Tig: tigecycline; Cs: Colistin; Tob: tobramycin; Net: netilmicin; Sxt: trimethoprim/sulfamethoxazole; Azt: aztreonam; Ptz: piperacillin/tazobactam; Ams: ampicillin/sulbactam; Min: minocycline.

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Phenotypic Relatedness of A. baumannii Isolates

The dendrogram of cluster analysis grouped the investigated *A. baumannii* isolates into three main segments shown in Figures 2, 3, and 4. Each segment consisted of a varying number of isolates spread out in clades and clusters.

The first segment (Figure 2) is a dendrogram showing the hierarchical clustering of seventy-six of the investigated isolates categorized into two clades with four clusters each. The grouping of the isolates did not show phenotypic relatedness in either their percentages of antibiotics-resistant patterns or sample types of the investigated isolates. However, in the first cluster of clade 1, some phenotypic relatedness in terms of percentage resistance (84.6%) of isolates 09 A61, 09 A86, and 09 A82 is seen as displayed in group 1 of Figure 2. Hence, clusters 2-4 comprising group B showed varied phenotypic non-relatedness, thus indicating diversity of isolates. Examining clade 2 and displayed clusters revealed that group K of cluster 8 showed phenotypical relatedness, exhibiting the same resistant profile from different sample sources. Other clusters (5-7) did not show any relatedness and, therefore, showed dissimilarities among the isolates.

The second (Figure 3) segment is the dendrogram clustering of twenty-two *A. baumannii* isolates of two clades having 5 clusters with four groups (A-D). Isolates clustered in groups A and B showed a somewhat phenotypical relatedness in terms of both resistance and isolate sources. Furthermore, isolate clusters in group D showed complete relatedness with percentage resistance

Table IV. Description of 2016-2018 *A. baumannii* isolates susceptible to four or more antimicrobials.

Bacterial isolate code	Resistance pattern	Susceptible antibiotics	Number sensitive	Percentage (%) susceptibility	Total
09 A6	MDR/CRAB	Tig, Cs, Tob, Net, Min	5	27.8	
09 A8	MDR/CRAB	Tig, Cs, Tob, Net, Min	5	27.8	
09 A12	SS	Tig, Cs, Imp, Mer, Cip, Levo	6	40.0	
09 A16	MDR	Tig, Cs, Imp, Amk	4	25.5	
09 A17	MDR/CRAB	Cs, Amk, Min, Sxt	4	22.2	
09 A26	MDR	Tig, Cs, Mer, Min	4	22.2	
09 A28	MDR/CRAB	Tig, Cs, Tob, Min	4	22.2	
09 A37	MDR/CRAB	Tig, Cs, Tob, Min	4	25.5	
09 A38	MDR/CRAB	Tig, Cs, Ams, Amk, Sxt	5	27.8	
09 A40	MDR/CRAB	Tig, Cs, Ams, Tob,	4	25.5	Total = 48
09 A41	MDR/CRAB	Tig, Cs, Amk, Net, Min	5	27.8	MDR = 4 (8.33 %)
09 A48	MDR/CRAB	Tig, Cs, Min, Sxt	4	22.2	SS = 5 (10.42 %)
09 A49	MDR/CRAB	Tig, Cs, Tob, Net, Min	5	27.8	MDR/CRAB = 39 (81.25 %)
09 A50	MDR/CRAB	Tig, Cs, Tob, Net, Min	5	27.8	
09 A51	MDR/CRAB	Tig, Cs, Tob, Net, Min	5	27.8	
09 A58	MDR/CRAB	Tig, Cs, Tob, Min	4	25.5	
09 A59	SS	Tig, Cs, Imp, Mer, Ams	5	41.7	
09 A64	MDR/CRAB	Tig, Cs, Tob, Min	4	22.2	
09 A65	MDR/CRAB	Tig, Cs, Tob, Min	4	22.2	
09 A66	SS	Tig, Cs, Imp, Mer, Pime, Caz	6	50	
09 A69	MDR/CRAB	Tig, Cs, Amk, Sxt	4	22.2	
09 A73	MDR/CRAB	Tig, Cs, Amk, Sxt	4	26.7	
09 A79	MDR/CRAB	Tig, Cs, Amk, Net, Min	5	27.8	
09 A87	MDR/CRAB	Tig, Cs, Amk, Net, Min	5	27.8	
09 A90	MDR/CRAB	Tig, Cs, Amk, Net.	4	26.7	
09 A91	MDR/CRAB	Tig, Cs, Net, Min	4	22.2	
09 A102	MDR/CRAB	Tig, Cs, Azt, Sxt	4	23.5	
09 A105	MDR/CRAB	Tig, Cs, Amk, Levo	4	26.7	
09 A108	MDR/CRAB	Tig, Cs, Amk, Min, Sxt	5	27.8	
09 A117	MDR/CRAB	Tig, Cs, Ams, Sxt	4	22.2	
09 A118	MDR/CRAB	Tig, Cs, Ams, Amk	4	28.6	
09 A124	MDR/CRAB	Tig, Cs, Amk, Net, Min	5	29.4	
09 A125	MDR/CRAB	Tig, Cs, Amk, Net, Min	5	27.8	
09 A127	MDR/CRAB	Tig, Cs, Tob, Net, Min	5	29.4	
09 A134	MDR/CRAB	Tig, Cs, Net, Pzt	4	26.7	
09 A142	MDR	Tig, Cs, Mer, Amk, Min, Sxt	6	33.3	
09 A143	MDR/CRAB	Tig, Cs, Amk, Min, Sxt	5	27.8	
09 A147	SS	Tig, Cs, Imp, Mer, Amk, Tob, Net, Min	8	47.0	
09 A150	SS	Tig, Cs, Imp, Mer, Amk, Tob, Net, Min	8	44.4	
09 A156	MDR/CRAB	Ams, Amk, Tob, Levo	4	25.5	
09 A157	MDR/CRAB	Tig, Cs, Tob, Net, Min	5	27.8	
09 A163	MDR/CRAB	Tig, Cs, Tob, Net,	4	22.2	
09 A164	MDR/CRAB	Tig, Cs, Amk, Net	4	30.8	
09 A169	MDR/CRAB	Tig, Cs, Net, Sxt	4	23.5	
09 A173	MDR/CRAB	Tig, Cs, Tob, Net, Min	5	27.8	
09 A174	MDR/CRAB	Tig, Cs, Tob, Net, Min	5	27.8	
09 A176	MDR/CRAB	Tig, Cs, Tob, Net, Min	5	27.8	
09 A178	MDR	Tig, Cs, Imp, Cip,	4	28.6	

MDR: multidrug-resistant; CRAB: carbapenem-resistant *Acinetobacter baumannii*; SS: susceptible strain; Imp: imipenem; Mer: meropenem; Amk: amikacin; Tig: tigecycline; Cs: colistin; Tob: tobramycin; Net: netilmicin; Ptz: piperacillin/tazobactam; Ams: ampicillin/sulbactam; Min: minocycline; Sxt: trimethoprim/sulfamethoxazole; Cip: ciprofloxacin.

Table V. Description of 2019-2020 *A. baumannii* isolates susceptible to antimicrobials.

Bacterial isolate code	Resistance description	Susceptible antibiotics	Number sensitive	Percentage (%) sensitive	Total
19 A04	XDR/CRAB	Sxt	1	10	
19 A14	MDR/CRAB	Tig, Sxt	2	11	
19 A16	MDR/CRAB	Tig, Amk, Tob, Net, Min	5	28	
19 A17	PDR/CRAB	None	0	0	
19 A20	XDR/CRAB	Sxt	1	10	Total Isolates = 28
19 A23	MDR/CRAB	Tig, Amk, Tob, Net, Min	5	28	
19 A29	MDR/CRAB	Tig, Amk, Tob, Net, Min	5	28	MDR/CRAB = 15 (53%)
19 A30	XDR/CRAB	NONE	0	0	XDR/CRAB = 10 (36%)
19 A34	MDR/CRAB	Tig, Amk, Tob, Net, Min	5	28	PDR/CRAB =3 (11%)
19 A36	PDR/CRAB	None	0	0	
19 A40	MDR/CRAB	Sxt	1	10	MDR/CRAB *vs.* XDR/CRAB $p = 0.0158$
19 A42	MDR/CRAB	Sxt	1	10	
19 A44	MDR/CRAB	Tig, Amk, Tob, Net, Min	5	28	
19 A45	MDR/CRAB	Tig	1	10	
19 A46	MDR/CRAB	Tig, Amk, Tob, Net, Min	5	28	
19 A50	MDR/CRAB	Sxt	1	10	
19 A55	MDR/CRAB	Sxt	1	10	
19 A57	MDR/CRAB	Tig, Amk, Tob, Net, Min, Sxt	6	33.3	
19 A58	XDR/CRAB	Tig	1	10	Comparison of proportion
19 A66	MDR/CRAB	Tig, Amk, Tob, Net, Min	5	28	
19 A68	PDR/CRAB	None	0	0	
19 A72	XDR/CRAB	Amk, Tob, Net	3	16.7	
19 A78	XDR/CRAB	Tig	1	10	
19 A 82	XDR/CRAB	Sxt	1	10	
19 A83	XDR/CRAB	Tig	1	10	
19 A88	XDR/CRAB	Tig	1	10	
19 A89	XDR/CRAB	Tig	1	10	
19 A91	MDR/CRAB	Tig, Amk, Tob, Net, Min	5	28	

MDR: multidrug-resistant; XDR: extensive drug-resistant; PDR: pan drug-resistant; CRAB: carbapenem-resistant *Acinetobacter baumannii*; Tig: tigecycline; Tob: tobramycin; Net: netilmicin; Sxt: trimethoprim/sulfamethoxazole; Amk: amikacin; Min: minocycline.

despite coming from different sources. The results indicate, therefore, that these isolates are likely of the same strain of *A. baumannii*.

In the third segment, a dendrogram of the phenotype relatedness analysis of (Figure 4) 79 *A. baumannii* isolates distributed them into two clades of nine (9) clusters, again showing diversity. The group B isolates in cluster 2 of clade 1 have ten isolates for which a similar percentage of resistance ranging from 81-89% was seen. Amongst these, 8 isolates displayed the same resistance profile, indicating that, a similar strain of *A. baumannii*. However, as observed in other *A. baumannii* isolates in previous figures, sample sources were different. Analysis of the dendrogram also in clade 1, cluster 3, revealed phenotypical relatedness for isolates 09 A6, 09 A51, 09 A125, 09 A157, 11 A158, 09 A173, 09 A174, and 09 A176, all with the same resistance profile of 72% (Figure 4).

However, for the isolates in group G of cluster 6, resistance was 81% (08 A3 and 08 A159) and 83% (09 A144, 09 A116, 09 A141). Also, two (2) isolates in this group (08 A74, 08 A33) had 87% resistance, while others (08 A42, 08 A43, 08 A114, 08 A119) exhibited 86% antimicrobial resistance. The similarity in this group (Group F) is attributed to the percentage of antimicrobial resistance (Figure 4). All other clusters in both clades 1 and 2 showed diversity in the strains of *A. baumannii,* as demonstrated by the phenotypical relatedness analyzed in **Supplementary Figure 1**, which shows a heatmap describing individual *A. baumannii* isolates mapping from these results.

Phylogeny Relatedness of Individual Isolates to Their Antimicrobial Susceptibility

Supplementary Figures 1, **2**, and **3** show a heatmap describing individual *A. baumannii*

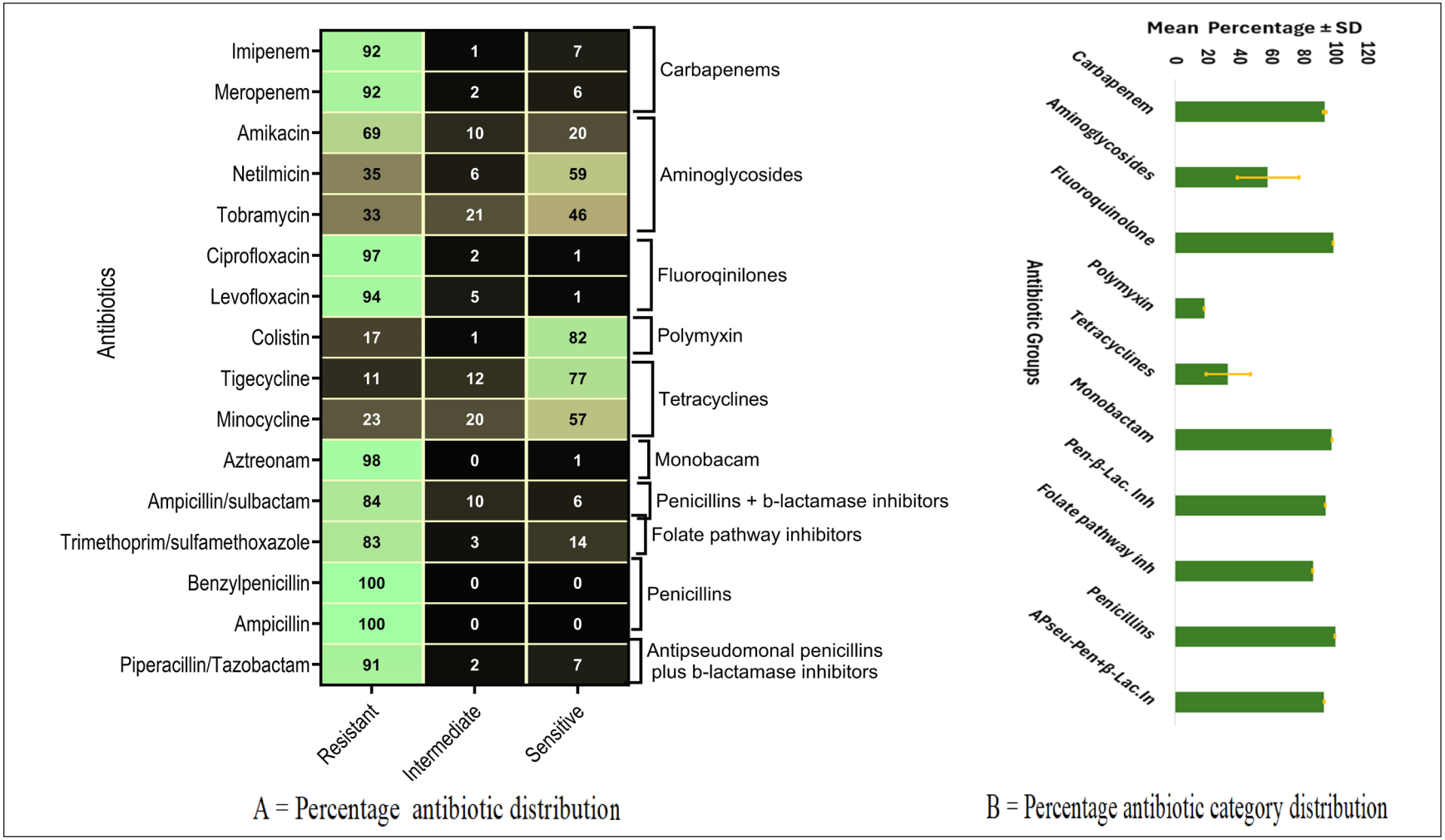

Figure 1. Heatmap showing the overall antimicrobial susceptibility of the investigated isolates against tested antibiotics (**A**) and the mean ± SD percentage resistance against antibiotic categories (**B**). Each box in panel (**A**) contains percentages for resistance, intermediate, and sensitivity values. Pen-β-Lac. Inh = Penicillins + β-lactamase inhibitors; Folate pathway inh = Folate pathway inhibitors; APseu-Pen+β-Lac. In = Antipseudomonal Penicillins plus β-lactamase inhibitors.

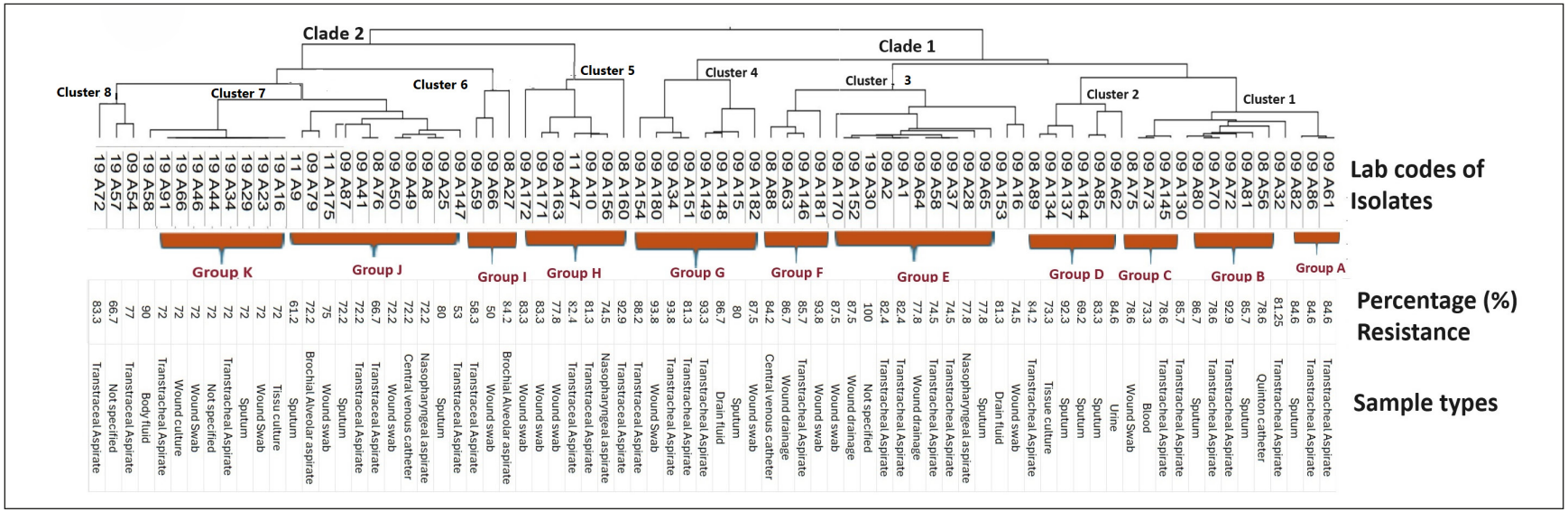

Figure 2. Dendrogram showing clusters of phenotypic relatedness amongst *A. baumannii* isolates. The first segment of the dendro gram of hierarchical clustering displays the codes of seventy-six of the investigated *A. baumannii* isolates, their resistance profile, and types of samples. Dendrogram is generated with Morpheus versatile matrix visualization and analysis software https://software.broadinstitute.org/morpheus.

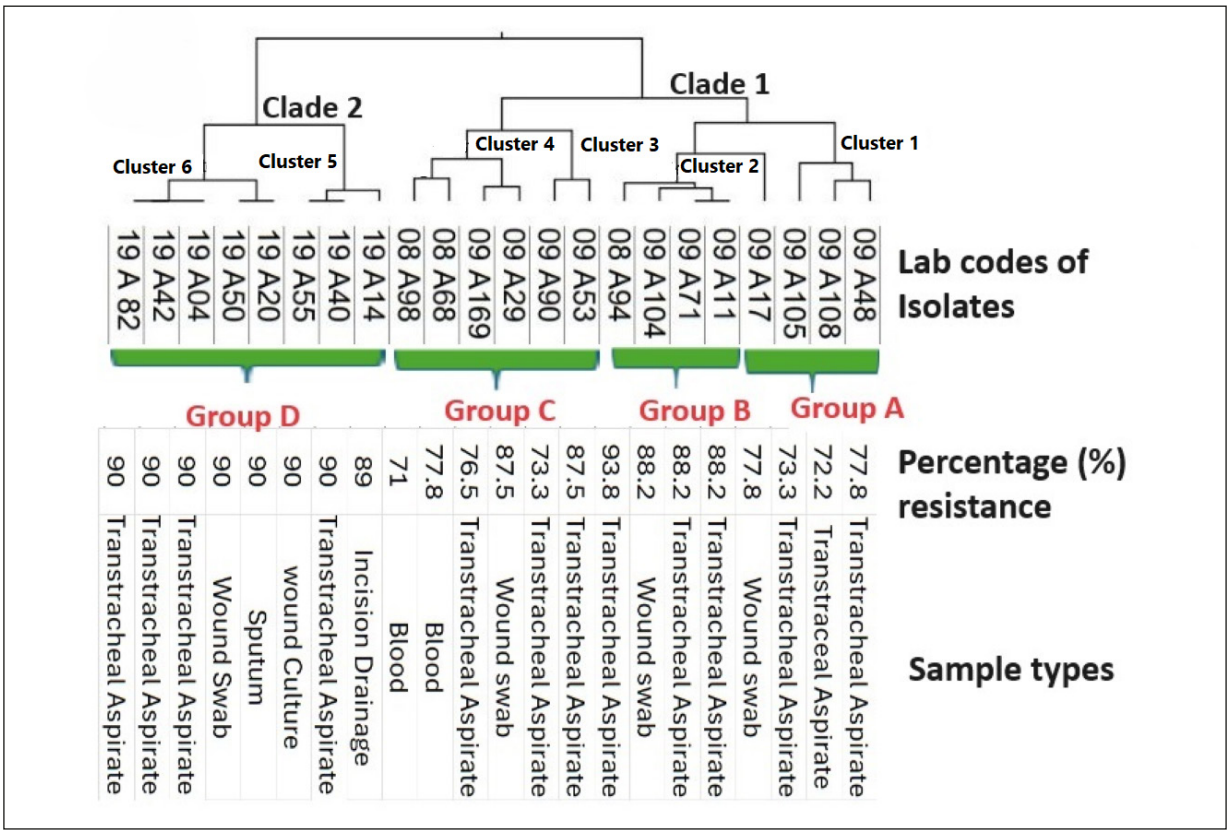

Figure 3. Dendrogram displaying clusters of *A. baumannii isolates* showing the extent of phenotypic relatedness. The second segment of the dendrogram of hierarchical clustering displays the codes of twenty-two of the investigated *A. baumannii* isolates, their resistance profile, and types of samples. Dendrogram is generated with Morpheus versatile matrix visualization and analysis software https://software.browadinstitute.org/morpheus.

isolates mapping to their antimicrobial assay based on the generated dendrogram of phenotype analysis. Hence, relating to their antibiotic susceptibility in in terms of sensitive, intermediate resistant, and resistant categories. Isolates 09 A56, 09 A81, 09 A72, 09 A70, and 09 A80 showed differences in the percentage of resistance but with similarities seen in their being resistant to some types of antibiotics (Imp, Mer Amk, amongst others) while being sensitive to Cs and the Tig (**Supplementary Figure 1**). However, two isolates in the group differed, with one (09 A72) showing intermediate susceptibility to Tig and the other (09 A56) resistant to Levo (**Supplementary Figure 1**).

Isolates 09 A11, 09 71, and 09 A104 (**Supplementary Figure 2**) showed phenotypical relatedness as documented earlier, therefore confirming their similarity; the heatmap showed that they were all sensitive to Sxt and Cs. They also displayed intermediate resistant characteristics to Tob, with only 09 A11 and 09 A71 showing intermediate resistance to Tig. Furthermore, phenotypic similarity of isolates 19 A14, 19 A40, 19 A55, 19 A20, 19 A04, 19 A42, and 19 A82 are confirmed with heatmap analysis, showing all being sensitive to Sxt, all of which were resistant to Cs while only one isolate (19 A14) exhibited sensitivity to Tig.

Trends in Time Distribution

The percentage distribution of resistance characteristics showed that a few isolates were PDR (Figure 5) while exhibiting very low sensitivity to other antimicrobials.

This figure also displays the frequency distribution of *A. baumannii* isolation and resistance characteristics demonstrated over the years investigated. Results showed that in the period of 2013-2014, *A. baumannii* isolates were more of MDR/CRAB. However, in the period 2016-2018, there was a reduction in *A. baumannii* isolates with MDR/CRAB, while some of the isolates

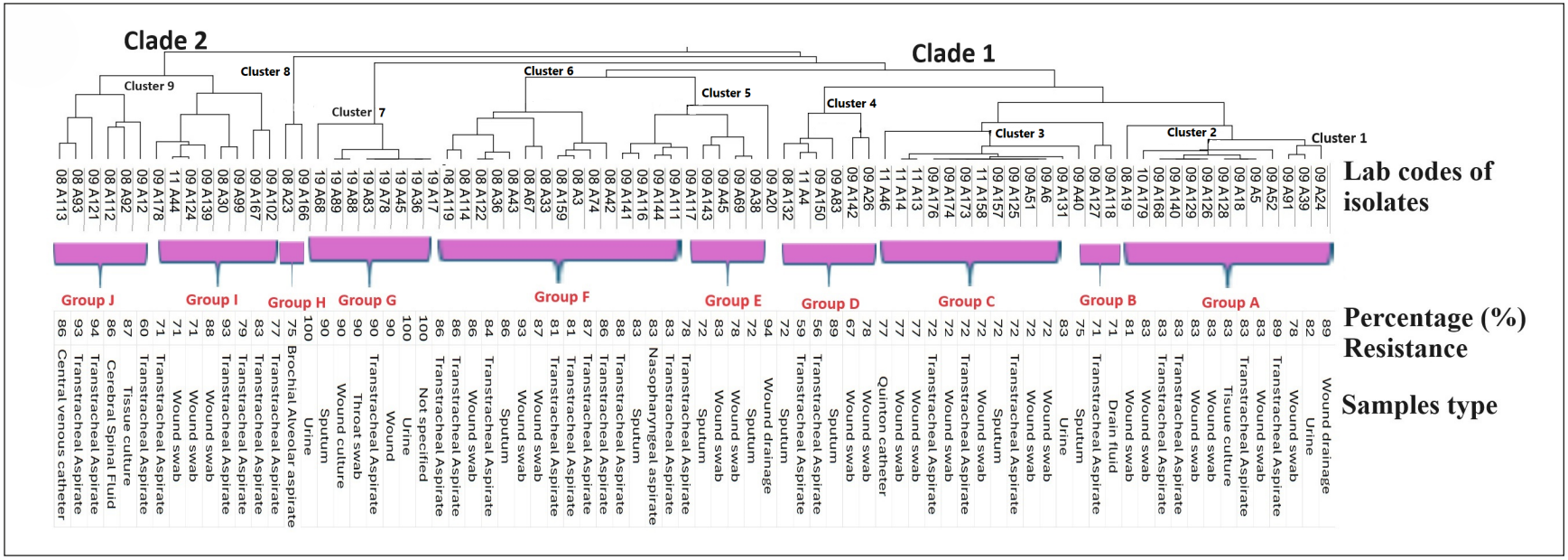

Figure 4. Dendrogram displaying clusters of *A. baumannii* isolates showing the extent of phenotypic relatedness. The third segment of the dendrogram of hierarchical clustering displays the codes of seventy-nine of the investigated *A. baumannii* isolates, their resistance profile, and types of samples. Dendrogram is generated with Morpheus versatile matrix visualization and analysis software https://software.broadinstitute.org/morpheus.

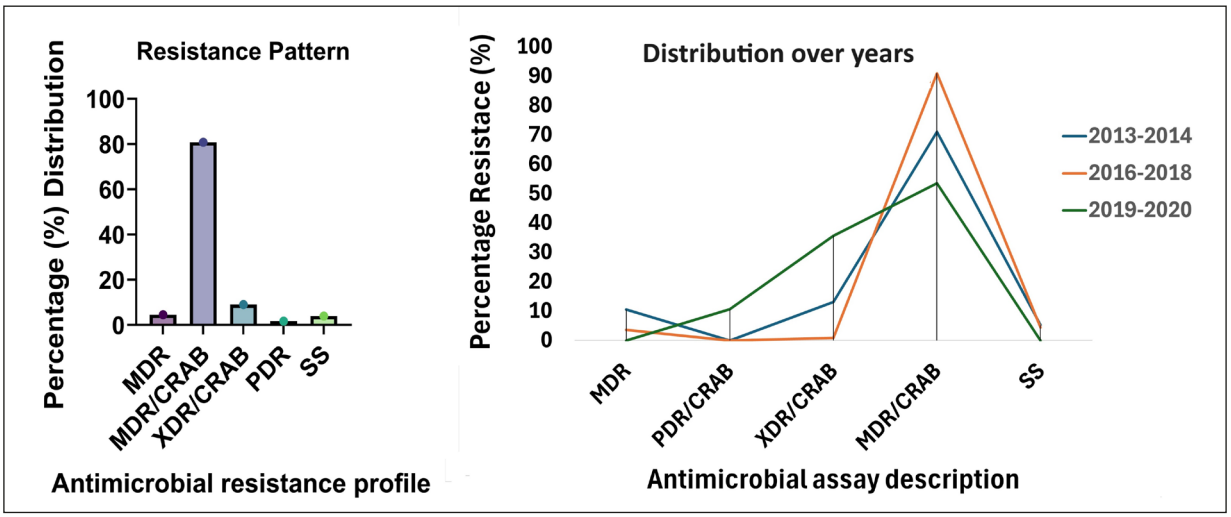

Figure 5. Describes resistance characteristics of *A. baumannii* isolates in relation to the number of antimicrobial categories and their distribution over year. Percentage antimicrobial resistance categories due to *A. baumannii* and periodic distribution display. MDR = multidrug resistance; CRAB = carbapenem resistant *A. baumannii*; XDR = extreme drug resistance; PDR = pan drug resistance; SS = susceptible strains.

exhibited XDR characteristics. Also, the period 2019-2020 produced MDR/CRAB, XDR/CRAB, and PDR isolate strains with no sensitive *A. baumannii* in this group.

Discussion

This report demonstrates that *Acinetobacter baumannii* is linked to hospitalized patients of various ages, consistent with other studies that identify the bacterium as an opportunistic pathogen frequently associated with nosocomial infections in critically ill patients[10,45]. The majority of patients were in the age range of 5-70 years old, which simply points to the fact that age is a risk factor and probably due to the fact that there are other comorbidities linked with this age group. Also, the sources of sample isolation are in accordance with those of other reports[45,46] on the ability to cause multi-system infections in patients. In addition, in conformity with the ongoing scientific discussion on the public health problem attributed to drug resistance by *A. baumannii* are the high levels of resistance profile displayed by the isolates in this study.

Most of the isolates here were MDR/CRAB while others were MDR, XDR/CRAB, or PDR *A. baumannii* (PDR-AB) strains and appear to be similar to those of previous reports, hence on the rise globally[47-49]. Worthy of note is the high incidence of resistance to carbapenem in this investigation. Carbapenem resistance by *A. baumannii* isolates in the region of this investigation has been reported extensively by researchers[17,18,40], all of which highlight the threat posed by this bacterium in constituting unprecedented significant challenges to clinicians in the management of critically ill patients.

The incidence rates of MDR/CRAB observed here are notably high and, in some cases, may even exceed those reported in other regions of the world[50]. Of the 177 *A. baumannii* isolates in this investigation, 162 (91.5%) of them were carbapenem resistant strains. This is contrary to the findings, which reported such strains to be in the range of between 1-30%[51,52] in Europe while placing those reported in the USA at 36-45%[40]. However, a greater than 85% average rate of resistance to carbapenem, as reported in Chain[50], could simply point to significant geographical differences. Again, the consistency in resistance rates observed over the years could be an indication of a public health problem that is not abating yet, thus presenting clinicians with limited treatment options. It is worth noting that the Middle East, including the region of this study, is reportedly one of the hardest hit by infections of *A. baumannii*[53], with travel from different regions of the world considered a contributory factor to antimicrobial resistance. There is also the case of *A. baumannii* intrinsically being resistant to some antibiotics, including penicillin, first and second generations of cephalosporins, and a wide range of other antimicrobials[28].

In this report, some isolates were seen to be sometimes sensitive to either one or two and at other times to three antibiotics, of which the most common were Tig and Cs. Previously, carbapenems had been the preferred treatment drug for MDR *A. baumannii*[54-56]. However, the rise of CRAB stains has led to the use of polymyxins, thus suggesting that the isolates here could have been treated with Cs. The polymyxins had previously been avoided for use in the treatment of XDR *A. baumannii* infections due to their neurotoxicity side effects[57]. Besides, the Infectious Disease Society of America (IDSA 2023) (https://www.idsociety.org/practice-guideline/amr-guidance/) discourages the use of Cs in treating CRAB infections in patients, while some researchers suggested the use of Cs in combined therapies[56]. Also, besides the toxicity of this last-line drug is the non-establishment of susceptible MIC breakpoints for both polymyxins and tigecycline, therefore suggesting ongoing work to sort this out. However, this will not stop the spread of resistance of *A. baumannii* to Cs as some isolates in this investigation were XDR and PDR *A. baumannii* strains, with the PDR strain being resistant to both Cs and Tig. Besides Cs, the isolates in this study were less resistant to the tetracyclines and tigecycline. Both of the antimicrobial agents are amongst those also recommended by IDSA for the management of CRAB infections. This is despite the fact that neither EUCAST, CLSI nor the US Food and Drug Administration (FDA) have provided breakpoints for tigecycline (CLSI 2014)[58].

Both XDR and PDR *A. baumannii* (PDR-AB) have been reported by other researchers[59-61]. Generally, reports on PDR-AB are on the increase globally[62], and as monotherapy is not an option for treating resultant infections, there are suggestions for the management of affected patients[63,64]. The use of cefiderocol as a last resort has been proposed[65]. However, due to the high level of hetero-resistance of this drug with polymyxins, it is recommended to explore alternative approaches for managing PDR-AB infections in patients[66,67].

The investigated *A. baumannii* isolates in this study displayed high phenotypic dissimilarities, revealing a wide range of different strains of this bacterium. The results are not unexpected due to the wide diffusion of *A. baumannii,* described as having an unrivaled adaptive nature in the acquisition of resistance markers[68]. The bacterium rapidly develops resistance to antimicrobials through several resistance mechanisms[28].

The results regarding the distribution of antimicrobial resistance over the years answer the question about any trends. MDR/CRAB were seen to remain consistently more than other resistance types throughout the period of observation. However, while there were no XDR nor PDR amongst the 2013-2014 isolates, XDR/CRAB were encountered in the 2016-2018 isolates. Also, while there was a percentage reduction in MDR/CRAB phenotypes among the 2019-2020 isolates, both PDRAB and XDR/CRAB were more in this period as compared to preceding years. These findings in the present report are similar to those of a recent report[69]. These findings align with reports indicating the increasing spread of antimicrobial resistance and the looming threat of a post-antibiotic era[39].

In this study, the diversity in resistance to antimicrobials by *A. baumannii* isolates shows that this opportunistic pathogen remains a public health threat, with an urgent need for new drugs and more infection control measures to mitigate the spread of CRAB in clinical settings. There is a need for improvements on existing therapeutics such as tigecycline and colistin with clearly defined measures for combined therapeutic procedures as the abating of the discussion of MDR *A. baumannii* is yet in sight.

Conclusions

The current study highlights that *A. baumannii* clinical isolates exhibited a diverse range of antimicrobial resistance, regardless of the sample source. This investigation also confirms that age might be a risk factor. The study shows a clear periodic trend in *A. baumannii* isolates changing resistance patterns from MDR to XDR and then to pandrug resistance over the study period. Also, it indicates that carbapenems might no longer be a treatment choice for *A. baumannii* infections in this study region. Although colistin exhibited less resistance, the toxicity of the drug reduces its usefulness. Hence, the development of pandrug resistance is a grave concern. Since *A. baumannii* is an opportunistic pathogen, it remains a public health threat, with an urgent need for new drugs and more infection control measures to mitigate the spread of CRAB in clinical settings.

Conflict of Interest
The authors declare there are no conflicts of interest.

Authors' Contributions
Conceptualization: L.I.B-E and P.M.E; methodology: L.I.B-E., S.A.Q; software: L.I.B-E and P.M.E; validation: L.I.B-E, S.A.Q and P.M.E; formal analysis: L.I.B-E. and P.ME; investigation: L.I.B-E., S.A.Q and P.M.E; resources: L.I.B-E.; data curation: L.I.B-E.; writing-original draft preparation: L.I.B-E writing-review and editing: L.I.B-E, S.A.Q and P.M.E; visualization: L.I.B-E and P.M.E; supervision: L.I.B-E.; funding acquisition: L.I.B-E. The manuscript has been read and approved for publication by all authors.

Ethics Approval
The Deanship of Scientific Research, under the Vice Presidency for Graduate Studies and Scientific Research, granted approval for the study, with the reference number KFU-REC-2024-FEB-ETHICS2017 (date of approval February 14th, 2024).

Informed Consent
Not applicable due to the design of the study.

Availability of Data and Materials
Data is available upon request from the authors.

AI Disclosure
The authors declare that AI tools were used for the writing of the manuscript.

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