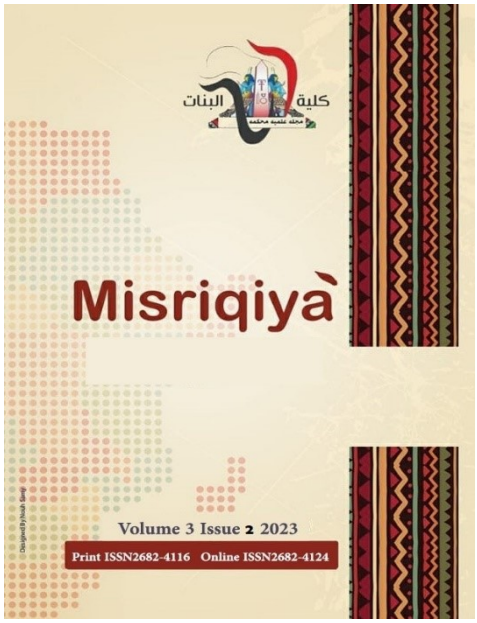

Phenotypic Screening of Multidrug-resistance (MDR) *Acinetobacter baumannii* from Public Hospitals in Egypt

Manal A. Adly[1,*], Shereen AH Mohamed[2], Soad A. Abdallah[1], Shaimaa S. Sobieh[1]

1: Botany Department, Faculty of Women for Arts, Science and Education, Ain Shams University, Cairo, Egypt.

2: Microbial Genetics Department, National Research Centre, Cairo, Egypt

Manalashraf02@gmail.com, shereen_asba@yahoo.com, soad.hussein@women.asu.edu.eg, shimaa.sobieh@women.asu.edu.eg

* Corresponding author

Received: 17-4-2023 Revised: 20-6-2023
Accepted: 25-6-2023 Published: 1-10-2023

DOI: 10.21608/MISJ.2023.209417.1046

Abstract

Multidrug-resistance (MDR) is a major and an on-going public health problem globally. It occurs mainly among Gram-negative pathogens of *Enterobacteriaceae* such as *Klebsiella pneumoniae, Pseudomonas aeruginosa* and recently observed in *Acinetobacter baumannii*, *Acinetobacter baumannii* are Gram-negative opportunistic bacteria with low virulence properties. Their resistance to antibiotics has become a problem of concern in hospital infections and one of the pathogens responsible for healthcare associated infections (nosocomial). A total of ١١٧ clinical samples of *Acinetobacter spp*. were collected through anonymous sources from the clinical microbiology laboratory and compartment or inanimate objects, and they were identified phenotypically 78 isolates were detected phenotypically as *A.baumannii* and 47/78 isolates (59.7%) were detected as MDR *A.baumannii*. This type of resistance are already widespread in certain parts of the world, particularly Europe, Asia and South America, while the situation in other places such as Africa is not well documented. This study aimed to investigate the multi-drug resistance profile of *A.baumannii* from different infection sites, and it's spreading in Egypt.

Keywords: *Multidrug-resistance (MDR), Acinetobacter baumannii, Hospital infection*

Introduction

Antibiotic resistance is a very current topic health concern and represents one of the most important challenges of the 21st century to human health due to extensive use over the last decades for this reason antibiotics are gradually losing their effectiveness **(Bondi et al., 2017).**

Acinetobacter baumannii is an important human pathogen belongs to the family Moraxellaceae, and is Gram-negative cocobacilli that is aerobic, pleomorphic, nonmotile, nonfermentative, oxidase-negative, and catalase-positive organism. It is known as opportunistic bacteria that have been reported in many infections last decades **(Bergogne-Berezin and Towner et al.,** 1996**).** Bacteremia, meningitis,

pneumonia, urinary tract infection, ventilator-associated pneumonia (VAP) and wound infections are the examples of *A. baumannii* infections.

A. baumannii is the most common species isolated from human clinical specimens, and present everywhere in the environment including soil, water and food, as well as in the hospital environment including ventilators, moisturizers, catheters and other medical equipment. The detection of *A. baumannii* infections in medical diagnostic laboratories of hospitals is usually carried out using phenotypic methods (including growth on MacConkey and blood agar media, and Gram staining) and differential biochemical tests, including oxidase, catalase, Oxidative-Fermentative (OF), Triple Sugar Iron (TSI), motility, Simon Citrate, methyl red (MR) and growth at temperatures of 37 °C and 44 °C **(Falah et al., 2019).** Today, different methods have been developed to better understand the epidemiology and clinical significance of *Acinetobacter* species as the Vitec analyzer Automated technology in microbiology has seen great advances in recent years. The choice of automated equipment for the identification and susceptibility testing of bacteria in a routine diagnostic laboratory depends on speed, accuracy, ease of use, and cost factors **(Shetty et al., 1998, Leylabadlo et al., 2015).**

A.baumannii was found to be resistant against antimicrobial drugs according to the Clinical and Laboratory Standards Institute (CLSI) guidelines 2022 **(Weinstein et al., 2020)**, such as aminopenicillins, cephalosporins, first and second generation cephalosporins, cephamycins, aminoglycosides, ureidopenicillins, chloramphenicol, and tetracyclines. Strains of *A.baumannii* have started to acquire resistance to newly developed antimicrobial drugs and become prevalent in many hospitals **(Howard et al., 2012).**

Acinetobacter baumannii strains resist all known antibiotics and are classified as PDR (Pan Drug Resistant) bacteria that should be characterized rapidly by the international health care community. Increase awareness and improvement of epidemiological surveillance is a significant and critical factor of successful infection control and is particularly recommended in hospitals. Hospitals have long served as reservoirs for the transmission of pathogenic bacteria, and this has become a problem. Research on the local population in Hospitals is important and beneficial to physicians, to help better diagnose and treat the infections, and prevents any outbreaks from spreading and to guide infection control policies in order to try and curb the spread of this bacterium **(Codjoe and Donkor et al., 2017).** Even though representative data are scarce, there is evidence for increasing incidence of *A. baumannii* outbreaks in healthcare facilities across Africa and Eastern Mediterranean countries, both of which have diverse populations **(Djahmi et al., 2014, Lowings et al., 2015)**. A comprehensive understanding of the current epidemiological picture is therefore needed. This will help with the development of context-specific prevention and control interventions against this dangerous and increasingly untreatable pathogen **(Ayobami et al., 2020).** In this study, we describe these developments as well as providing a comprehensive assessment of microbiological and epidemiological characteristics *A. baumannii*, and to determine the prevalence of multidrug-resistant *A. baumannii* in public hospitals in Egypt.

Materials and methods

Sample collection

Samples were collected anonymously from clinical microbiology laboratories of different public hospitals. The samples were collected from different compartments (ICU, wards, surgery rooms) as well as from inanimate sources including bed rails, ventilators, monitors…etc.). A total of 117 isolates were confirmed as *Acinetobacter* species.

Phenotypic identifications of isolate

All isolates were confirmed as *A. baumannii* based on morphological tests (colony morphology and Gram staining results) and biochemical tests including catalase **(Reiner 2013)**, urease **(Konieczna et al. 2012)**, oxidative-fermentative medium (OF) **(Hall et al., 1972),** oxidase **(Shields and Cathcart 2010)**,triple sugar iron agar (TSI) **(Lehman 2005)**, citrate **(MacWilliams 2009)**, SIM tests **(Dalynn Biologicals 2011)**, and confirmed by API 20E multi test system (Biomerieux ,France). These tests were used according to manufacturer's protocol for *Enterobacteriaceae* and nonenteric bacteria. Wells of biochemical test were inoculated with overnight 0.5 McFarland bacterial suspensions and incubated at 37º C for 24 hrs. The results were read after addition of reagents, as 7 digit number that identified by API 20 analytical index, and confirmed as *A. baumannii* by the automated method VITEK 2 Compact (Biomerieux ,USA), VITEK 2 is a fully automated system that performs bacterial identification and antibiotic susceptibility testing**(Pincus 2013)**. The isolates were stored in the MacConkey broth medium with 40% glycerol at -80 °C for further analysis.

Antibiotic susceptibility test

Pattern of antimicrobial resistance was studied using the simple disk diffusion technique **(Hudzicki 2012) .** All isolates were subjected to antibiotic susceptibility test, performed on Muller-Hinton agar (MHA) plates by the kirby-bauer disc diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI) guidelines 202٢. Nine antibiotics including aztreonam (monobactam) (30 µg/disk); amikacin (aminoglycoside) (30 µg/disk); ciprofloxacin and levofloxacin (quinolone) (5 µg/disk); cefepime (cephalosporin) (30µg/disk); cefotaxime (cephalosporin) (30 µg/disk); imipenem (carbapenem) (10µg/disk; piperacillin/tozabactam (penicillins) (10 µg/disk); colistin (polymyxin) (10 µg/diskwere used to determine the multidrug-resistant MDR status of *A. baumannii*. All of the inoculated plates were aerobically incubated at 42°C for 18-24h in an aerobic atmosphere. Results were interpreted based on the instructions provided by CLSI (2022).

Results

Phenotypic identifications of isolate and susceptibility to antibiotic

One hundred and seventeen isolates were collected through anonymous sources from the clinical microbiology laboratory and compartment or inanimate objects (fomites, annual wheel chairs, beds, floors) and other medical equipment (ventilators, moisturisers, catheters, nebulizers). Only 78 isolates (66.6%) were identified as suspected *A.baumannii* based on the morphological and biochemical characteristics, has been carried out according to Berger's manual of systematic bacteriology **(Sneath et al., 1986)**. Results showed biochemical identification for the most patent bacterial isolates using VITEK2 system , VITEK card contains 96 wells,

with physiological and biochemical tests. The colonies were short and large Gram-negative coccobacilli appear to be diplococci-like. On blood agar, colonies were smooth, clear to matt without any hemolysis and non-pigmented, with smooth-to-pitted surfaces in a diameter of 1-2mm. On MacConkey agar, colonies of *A. baumannii* were non-lactose fermenter, pure purple and mucoid. All strains of *A. baumannii* were found to be catalase positive and oxidase/indole negative. Triple sugar iron agar (TSI) test had alkaline bottom/alkaline slant, lack of gas production and H_2S. Oxidative-fermentative medium (OF) test had acid production of glucose in aerobic conditions. Sulphur, indole, and motility tests (SIM) showed immobility, lack of both Indol, and H_2S production (Table.1). Isolates grew at 37 °C - 44 °C and the optimum growth was at 42°C.

Isolates were confirmed as *A. baumannii* by conventional analytical profile index API20E system (Biomerieux, France) code (0204042) (Fig. 1), and confirmed as *A. baumannii* by using VITEK 2 Compact

Table (1) Phenotypical and biochemical characterization of *A. baumannii* isolates using VITEK test.

Biochemical tests	Results
Macroscopic characterization of colonies on blood-agar and MacConkey agar mediums	Blood-agar medium: smooth, clear to matt colonies, without any hemolysis and pigmentation MacConkey agar medium: Pure purple and mucoid colonies
Microscopic properties of growth colonies	Short and obese Gram-negative coccobacilli appear to be diplococci-like
Catalase and oxidase tests	Catalase positive, negative oxidase
TSI, OF, SIM tests	TSI: Alk/Alk, lack of gas production and H2S OF: Acid production of glucose in aerobic conditions SIM: Immobility, lack of Indol, and H2S production
Growth at temperatures of 37 °C and 44°C	Growth at temperatures of 37 °C and 44 °C optimum at 42°C

Fig. 1. Result of *A. baumannii* by API 20E system (Biomerieux, France).

Antibiotic sensitivity patterns of the isolates were determined by the disk diffusion method, as described by the National Committee for Clinical Laboratory Standards (CLSI) 2022, with commercial antimicrobial disks (Oxoid,U.K). Antibiotic susceptibility results showed high drug resistant *A. baumannii* isolates that reached 59.7% (47/78) and were determined as MDRs. *A. baumannii* isolates showed the highest resistance for aztreonam (monobactam); amikacin (aminoglycoside); ciprofloxacin ; levofloxacin (quinolone); cefepime and cefotaxime (cephalosporin) , imipenem , meropenem (carbapenem), piperacillin/tozabactam and colistin (Table. 2).

Table (2): Antibiotic susceptibility profiles of multi-drug resistant *A. baumannii* isolates

Serial no	NO of isolate	Antibiotic resistant pattern by disk diffusion method
1	1	AZM,AK, LEV, CIP, FEP, CTX, IMP, PTZ, CT.
2	3	AZM,AK, LEV, CIP, FEP, CTX, IMP, PTZ, CT.
3	4	AZM,AK, LEV, CIP, FEP, CTX, IMP, PTZ, CT.
4	5	AZM,AK, LEV, CIP, FEP, CTX, IMP, PTZ, CT.
5	8	AZM,AK, LEV, CIP, FEP, CTX.
6	10	AZM,AK, LEV, CIP
7	11	AZM,AK, LEV, CIP, FEP, CTX, IMP, PTZ, CT.
8	14	AZM,AK, LEV, CIP, FEP, CTX, IMP, PTZ, CT.
9	16	AZM,AK, LEV, CIP
10	18	AZM,AK, LEV, CIP, FEP, CTX.

11	**19**	AZM,AK, LEV, CIP, FEP, CTX.
12	**21**	AZM,AK, LEV, CIP, FEP, CTX, IMP, PTZ, CT.
13	**26**	AZM,AK, LEV, CIP
14	**27**	AZM,AK, LEV, CIP
15	**30**	AZM,AK, LEV, CIP, FEP, CTX, IMP, PTZ, CT.
16	**32**	AZM,AK, LEV, CIP, FEP, CTX.
17	**39**	AZM,AK, LEV, CIP
18	**42**	AZM,AK, LEV, CIP, FEP, CTX, IMP, PTZ, CT.
19	**48**	AZM,AK, LEV, CIP, FEP, CTX.
20	**51**	AZM,AK, LEV, CIP
21	**52**	AZM,AK, LEV, CIP
22	**53**	AZM,AK, LEV, CIP, FEP, CTX.
23	**55**	AZM,AK, LEV, CIP
24	**60**	AZM,AK, LEV, CIP, FEP, CTX.
25	**62**	AZM,AK, LEV, CIP
26	**65**	AZM,AK, LEV, CIP, FEP, CTX.
27	**67**	AZM,AK, LEV, CIP
28	**69**	AZM,AK, LEV, CIP
29	**72**	AZM,AK, LEV, CIP, FEP, CTX.
30	**75**	AZM,AK, LEV, CIP
31	**77**	AZM,AK, LEV, CIP
32	**78**	AZM,AK, LEV, CIP, FEP, CTX, IMP,PTZ, CT.
33	**80**	AZM,AK, LEV, CIP, FEP, CTX.
34	**82**	AZM,AK, LEV, CIP
35	**85**	AZM,AK, LEV, CIP, FEP, CTX.
36	**86**	AZM,AK, LEV, CIP
37	**90**	AZM,AK, LEV, CIP, FEP, CTX, IMP, PTZ, CT.
38	**93**	AZM,AK, LEV, CIP, FEP, CTX.
39	**96**	AZM,AK, LEV, CIP
40	**99**	AZM,AK, LEV, CIP
41	**103**	AZM,AK, LEV, CIP, FEP, CTX.
42	**105**	AZM,AK, LEV, CIP
43	**107**	AZM,AK, LEV, CIP, FEP, CTX.

44	**109**	AZM,AK, LEV, CIP
45	**112**	AZM,AK, LEV, CIP, FEP, CTX.
46	**114**	AZM,AK, LEV, CIP
47	**115**	AZM,AK, LEV, CIP, FEP, CTX.

Discussion

Multi-drug resistance (MDR) clinically, is the ability of disease causing microorganism to withstand a wide variety of antimicrobial compounds **(Kennedy et al., 2009).** Hence, a strain is considered a MDR if an isolate is resistant to representatives of three or more classes of antibiotics. However, transference of resistance determinants by mobile genetic elements including plasmids, transposons, and gene cassettes in integrons between and across different bacterial species are important factors that can contribute to the increase in multi-resistant strains **(Livermore et al., 2007).** Based on this observation, the ability of *A. baumannii* isolates for fully resistant to antibiotics could be caused by either; predominate exposure of present isolates to suboptimal levels of antibiotic, prolong use of broad-spectrum antibiotics, exposure to isolates carrying resistant genes, lack of hygiene in clinical environments and usage of antibiotics in foods and agriculture.

Acinetobacter baumannii is one of the main causes of healthcare associated infections in recent years **(Zhou et al,. 2018**) and is difficult to control due to the prevalence of multiple drug resistance microorganisms in hospitals. Nosocomial infections caused by the strains of these bacteria are increasingly becoming resistant to a range of antibiotics.

In this study, the determination of *A. baumannii* isolates, that were collected and identified phenotypically and biochemically, and compared the results with those of the automated method VITEK 2 Compact to confirm *A. baumannii* isolates.

VITEK 2 system was used for authenticating names of *A. baumannii* as described by the manufacturer (bioMerieux Inc., Durham, NC 27712, USA). VITEK 2 machine controlled the card automatically including the filling, sealing, and then transferring the cards into the linked incubator (35°C). Each output report is decoded according to a particular algorithmic system. The acquired results were compared to the ID-GN (identifcation of Gram-negative bacteria) databank.

Most studies have been conducted to evaluate the antibiotic susceptibility patterns in clinical strains of *A. baumannii* **(Liu et al., 2018).** In the present study *A. baumannii* isolates were detected as MDRs that are resistant to all examined antibiotics. There was a 59.7% *A.baumannii* isolates were detected as MDR-*A.baumannii* isolates that showed the highest resistance for aztreonam and piperacillin/tozabactam and the highest resistance for imipenem and meropenem and the highest resistance for colistin in the collected samples. When comparing these results with those of previously published studies **(Köck et al., 2013, Lowings et al., 2015)** the results indicate that the problem will increase. The problem is the increased number of antibiotic resistance and predominance of MDR *A. baumannii* since 2008 in Africa (Egypt, South Africa). The percentage of resistance increased dramatically from 2008 till now. Resistance to these antimicrobials might be likely to increase in the coming years if they are not used as prescribed by national and international

guidelines **(Sekyere et al., 2016).** A comprehensive understanding of the current epidemiological picture is therefore needed. This will help with the development of context-specific prevention and control interventions against this dangerous and increasingly untreatable pathogen **(Ayobami et al., 2020).**

.

Conclusions

This study outlines the relevance of hospital-acquired infections caused by *A. baumannii*, especially MDR-resistant strains, in Africa (Egypt, South Africa), Mediterranean WHO regions. A higher rate of MDR-resistant *A. baumannii* was shown as an important health issue. Such a higher spread now it is considered a new threat in clinical settings, which it requires a great stop to reduction its prevalence, the strains also indicated significant challenges for healthcare organizations, public health, and the elderly. Identification of *A. baumannii* isolates as MDRs that are resistant to the entire examined antibiotic was performed.

Author Contributions: All authors suggested the idea, the objective and designed the plan of the work. Moreover, they provided constructive suggestions for the analysis of the results and discussion. They all participated in the manuscript writing.

References

Ayobami, O., Willrich, N., Reuss, A., Eckmanns, T., & Markwart, R. (2020). The ongoing challenge of vancomycin-resistant Enterococcus faecium and Enterococcus faecalis in Europe: an epidemiological analysis of bloodstream infections. *Emerging Microbes & Infections*, 9(1), 1180-1193.

Bergogne-Berezin, E., & Towner, K. J. (1996). Acinetobacter spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. *Clinical microbiology reviews*, *9*(2), 148-165.

Bondi, M., Mazzini, A., De Niederhäusern, S., Iseppi, R., & Messi, P. (2017). Inhibition of multidrug-resistant Gram-positive and Gram-negative bacteria by a photoactivated porphyrin. *Polish Journal of Microbiology*, *66*(4), 533-536.

Codjoe, F. S., & Donkor, E. S. (2017). Carbapenem resistance: a review. *Medical Sciences*, *6*(1), 1.

Dalynn Biologicals. 2011. "Sulfide Indole Motility Medium." Sulfide Indole Motility Medium (Sim): 2–3. http://www.microbelibrary.org

Djahmi, N., Dunyach-Remy, C., Pantel, A., Dekhil, M., Sotto, A., & Lavigne, J. P. (2014). Epidemiology of carbapenemase-producing Enterobacteriaceae and Acinetobacter baumannii in Mediterranean countries. *BioMed research international*, *2014*.

Falah, F., Vasiee, A., Behbahani, B. A., Yazdi, F. T., Moradi, S., Mortazavi, S. A., & Roshanak, S. (2019). Evaluation of adherence and anti-infective properties of probiotic Lactobacillus fermentum strain 4-17 against Escherichia coli causing urinary tract infection in humans. Microbial pathogenesis, 131, 246-253.

Hall, C.T., Webb, D. & Papageorge, C. Use of an oxidation-fermentation medium in the identification of yeasts. HSMHA Health Reports. 87(2), 172–176 (1972).

Howard, A., O'Donoghue, M., Feeney, A., & Sleator, R. D. (2012). *Acinetobacter baumannii*: an emerging opportunistic pathogen. Virulence, 3(3), 243-250.

Hudzicki, J. Kirby-Bauer disk diffusion susceptibility test protocol author information. Americ. Soci. Microbiol.1–13 (2012).

Kennedy, J., Baker, P., Piper, C., Cotter, P. D., Walsh, M., Mooij, M. J., & Dobson, A. D. (2009). Isolation and analysis of bacteria with antimicrobial activities from the marine sponge *Haliclona simulans* collected from Irish waters. Marine biotechnology, 11, 384-396.

Köck, R., Schaumburg, F., Mellmann, A., Köksal, M., Jurke, A., Becker, K., & Friedrich, A. W. (2013). Livestock-associated methicillin-resistant Staphylococcus aureus (MRSA) as causes of human infection and colonization in Germany. *PloS one*, *8*(2), e55040.

Konieczna, I., Zarnowiec, P., Kwinkowski, M., Kolesinska, B., Fraczyk, J., Kaminski, Z., & Kaca, W. Bacterial urease and its role in long-lasting human diseases. Curr. Prot. Pept. Sci. 13(8), 789-806 (2012).

Lehman, D. Triple sugar iron agar protocols. Microb. Librar. (2005).

Leylabadlo, H. E., Asgharzadeh, M., & Aghazadeh, M. (2015). Dissemination of carbapenemases producing Gram negative bacteria in the Middle East. *Iranian journal of microbiology*, *7*(5), 226.

Liu, Y., Li, R., Xiao, X., & Wang, Z. Molecules that inhibit bacterial resistance enzymes. Molecul. 24(1), 43 (2018).

Livermore, D. M., Canton, R., Gniadkowski, M., Nordmann, P., Rossolini, G. M., Arlet, G., ... & Woodford, N. (2007). CTX-M: changing the face of ESBLs in Europe. Journal of Antimicrobial Chemotherapy, 59(2), 165-174.

Lowings, M., Ehlers, M. M., Dreyer, A. W., & Kock, M. M. (2015). High prevalence of oxacillinases in clinical multidrug-resistant Acinetobacter baumannii isolates from the Tshwane region, South Africa–an update. BMC infectious diseases, 15(1), 1-10.

MacWilliams, M. P. (2009). Citrate test protocol. *American Society for Microbiology*

Pincus, D. H. (2006). Microbial identification using the bioMérieux Vitek® 2 system. *Encyclopedia of Rapid Microbiological Methods. Bethesda, MD: Parenteral Drug Association*, *2006*, 1-32.

Reiner, K. 'Catalase Test Protocol', (November 2010), 1–9 (2013).

Sekyere, J. O., Govinden, U., & Essack, S. (2016). The molecular epidemiology and genetic environment of carbapenemases detected in Africa. Microbial drug resistance, 22(1), 59-68.

Shetty, P. G., Shroff, M. M., Sahani, D. V., & Kirtane, M. V. (1998). Evaluation of high-resolution CT and MR cisternography in the diagnosis of cerebrospinal fluid fistula. American Journal of Neuroradiology, 19(4), 633-639.

Shields, P. (2010). Cathcart L. Oxidase test protocol. American Society for Microbiology.Sneath, P. H. (1986). Endospore-forming Gram-positive rods and cocci. *Bergey's manual of systematic bacteriology*, *2*, 1104-1207.

Weinstein, M. P., & Lewis, J. S. (2020). The clinical and laboratory standards institute subcommittee on antimicrobial susceptibility testing: background, organization, functions, and processes. *Journal of clinical microbiology*, *58*(3), e01864-19.

Zhou, Y., Wang, T., Guo, Y., Liu, S., Wang, J., Shen, Y., ... & Deng, X. (2018). In vitro/vivo activity of potential MCR-1 inhibitor in combination with colistin againsts mcr-1-positive Klebsiella pneumonia. *Frontiers in Microbiology*, *9*, 1615.