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Clinical Evidence of Multi-drug Resistant, Extensively Drug Resistant and Pan-drug Resistant *Acinetobacter* sp. in Bangladesh

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Abstract

Blood borne infections are one of the major health problems in Bangladesh, which requires frequent use of antimicrobials. In most of the cases, diagnosis and treatment with such antimicrobials are done empirically. Therefore, updated information on etiological data for major pathogens and their pattern of antibiotic resistance is required for formulating strategy and guideline for the prescribers. This study was carried out to assess the pattern of antibiotic resistance and to determine the prevalence of resistance phenotypes of different bacterial pathogens, including *Acinetobacter* sp. previously collected from patients from a hospital in Dhaka, Bangladesh. Retrospective analysis of 573 clinical records was performed, and the antimicrobial susceptibility pattern of those records was used to determine the prevalence of the isolates that were multi-drug resistant. Our finding indicates that *Acinetobacter* sp. (12%), followed by *Salmonella typhi* (58%) were the second most often found pathogen in the blood samples. In addition, we identified that these organisms exhibited a multi-drug resistance (MDR) pattern toward the most frequently used antibiotic classes, including cephalosporin, fluoroquinolones, aminoglycosides and carbapenems. We found moderate to high levels of resistance against aminoglycosides (45-53%), cephalosporins (28-45%), fluoroquinolones (28-39%) and carbapenem (17-19%) in *Acinetobacter* sp. (11.7%, n = 67), as well as multi-drug resistant (66.7%, n = 38), and extensively drug-resistant or XDR (13.64%, n = 9), isolates. We also found pan-drug resistant (PDR) isolates (2.3%) of *Acinetobacter* sp. showing resistance against all antibiotics that are used clinically. In order to launch effective treatment strategies and prevent the further emergence of MDR, it can be suggested that extensive national antimicrobial surveillance be conducted against these pathogens. There is also a need for further characterization of such superbugs to address the issue of antimicrobial resistance.

Key words: Antimicrobials, *Salmonella typhi*, *Acinetobacter* sp., multi-drug resistance (MDR), carbapenems, extensively drug-resistant (XDR), pan-drug resistant (PDR).

Introduction

Blood stream infections are one of the most common causes of mortality and morbidity and the most common healthcare associated infections (Diekema *et al*., 2003). Illness associated with blood stream infection can range from self-limiting infections to life-threatening sepsis that requires rapid and aggressive antimicrobial treatment. A wide spectrum of organisms is responsible for such infections and this variation in spectrum as well as their resistance profile is dependent greatly upon geographical alteration. In almost all cases, antimicrobial therapy is initiated empirically before the results of blood culture are available (Gohel *et al*.,

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2014). However, rampant use of antibiotics is responsible for increasing antimicrobial resistance, which is now a worldwide concern. The prevalence of resistance in both children and elderly patients is increasing, and it varies greatly in accordance with geographical and regional location (Lawrence and Jeyakumar, 2013; Prestinaci *et al.*, 2015). Therefore, the study of antibiotic resistance patterns from blood culture isolates, can be a useful guideline for clinicians initiating the empiric antibiotic therapy (Gohel *et al.*, 2014).

Antimicrobial resistance (AMR) is now-a-days a serious health issue for the rapid emergence of multidrug resistant (MDR) clinical isolates. MDR is defined as non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos *et al*., 2012). In addition to the emergence of MDR clinical isolates, the recent global outbreak of extensively drug-resistant (XDR) and pandrug-resistant (PDR) clinical isolates has further intensified the problem of AMR. These are sometimes referred to as the superbugs, among which include the MDR *Acinetobacter* sp. bacteria that pose antibiotic-resistant threats to patients in healthcare settings. The *Acinetobacter* species is a common and major cause of nosocomial infections (Towner *et al*., 2009) and possible reasons of their emergence might be due to survival ability and quicker development of resistance to the major available antibiotic classes (Towner *et al*., 1997). Multi-antibiotic resistant *Acinetobacter baumannii*, is now recognized to be of great clinical significance. Numerous reports rely on the spread of *A. baumannii* in the hospital settings which leads to enhanced nosocomial outbreaks associated with high death rates (Almasaudi, 2018; Wong *et al.*, 2017). There is scarcity of data on the clinical evidence of *Acinetobacter* sp. in our country. The major objective of the study was to find out the frequency of *Acinetobacter* sp. among various blood borne pathogens causing infections in the country. We also intended to study the antibiotic sensitivity and resistance pattern of *Acinetobacter* sp., and to investigate the occurrence of MDR, XDR and PDR among them.

Materials and Methods

This was a single-center, retrospective study on the sensitivity pattern of various pathogenic bacteria (n = 573) including *Acinetobacter sp.* against empirically used conventional antibacterial agents. The sensitivity pattern was deciphered by retrieving the clinical record of the antibiogram (January 2018 - June 2018) from the Microbiology Laboratory, Popular Diagnostic Center, House No. 16, Rd No. 2, Dhaka 1205, Bangladesh. The antibiogram contained the information of sensitivity or resistance of various pathogens against antibiotics that are used empirically. Prior to antibiogram, the pathogens were isolated, identified and characterized in the microbiology laboratory of the hospital in accordance with standard microbiological procedures (Clinical and Laboratory Standards Institute, 2020), as confirmed by the laboratory technician. The antibiotic sensitivity was checked by performing the disk diffusion method (Bauer *et al.*, 1966). The antibiogram data were generated from the blood culture positive clinical isolates collected previously from the suspected patients. It also contained information about the date of receiving the sample, the delivery date of result and the specimen used for the tests. For ease of the data collection, a data collection form was prepared (not shown). This form included test results showing the presence of pathogenic microorganisms and their sensitivity against a panel of conventionally used antibacterial agents. The sensitive, resistant and the intermediate resistant pathogens as defined according to the CLSI guideline (Clinical and Laboratory Standards Institute, 2020) were designated and codified as ‘S’, ‘R’ and ‘M’, respectively. Prior to data collection, ethical permission was taken from the institutional ethical committee of Popular Diagnostic Center, House 16, Road 2, Dhanmondi, Dhaka-1205, Bangladesh. The data were entered into Microsoft Excel and then examined for the pathogen kind and sensitivity pattern. The frequency of MDR, XDR and PDR pathogens was estimated using the sensitivity pattern. As stated in the introduction, the organisms showing non-susceptibility to at least one agent in three or more antimicrobial categories were identified

as MDR, whereas non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates showing susceptibility to only one or two categories) were grouped as XDR and yet the organisms exhibiting non-susceptibility to all agents in all antimicrobial categories were counted as PDR (Magiorakos *et al.*, 2012). Since bacterial isolates were tested against nearly all of the antimicrobial agents within the antimicrobial categories, the MDR, XDR and PDR isolates could be identified and estimated as suggested by the Centers for Disease Control and Prevention (CDC) and the European Centre for Disease Prevention and Control (ECDC) without any bias of using select antibacterial agents (Magiorakos *et al.*, 2012).

Results and Discussion

High prevalence of Acinetobacter sp. *among the MDR pathogens:* A total of 573 isolates were collected, among which 5 was of *Streptococcus pneumoniae*, 8 was of *Enterococcus* sp., 8 was of *Staphylococcus aureus*, 11 was of *Pseudomonas aeruginosa*, 33 was of *Klebsiella pneumoniae*, 50 was of *Escherichia coli*, 66 was of *Acinetobacter* sp., 58 was of *Salmonella paratyphi*, 334 was of *Salmonella typhi* (Table 1). Most of these clinical isolates, except *E. coli* and *Salmonella* sp., belonged to the ESKAPE group of highly resistant pathogens responsible for nosocomial infections (Davin-Regli *et al.*, 2019; Pandey et al., 2021). Of these isolates, *Acinetobacter* sp. was found to be the second most prevalent (12%) resistant pathogens with 79% of MDR cases (Table 1). Like other members of the ESKAPE family, *Acinetobacter* sp., has been reported to show high level of antimicrobial resistance (AMR) against most of the available antibacterial agents and this is attributable to various resistance mechanism including inactivation and/or modification of the drugs or their binding sites or targets, alteration in the cellular permeability and by other mutational events (Pandey *et al.*, 2021; Kyriakidis *et al.*, 2021).

Table 1. Distribution of pathogens and their MDR* isolates.

Pathogens	Percentage of isolates	% MDR
Streptococcus pneumoniae	1%	100.0
Enterococcus sp.	1%	87.5
Klebsiella pneumoniae	6%	84.9
Escherichia coli	9%	82.0
Acinetobacter sp.	12%	78.8
Staphylococcus aureus	1%	37.5
Salmonella typhi	58%	22.8
Pseudomonas aeruginosa	2%	9.1
Salmonella paratyphi	10%	1.7

*MDR isolates were those showing resistance against at least three or more classes of antibiotics

Presence of resistant Acinetobacter sp. against most of the empirically used antibiotics: Among the various antibiotics used against *Acinetobacter* sp. includes those from penicillin, cephalosporin, macrolide, fluoroquinolone, aminoglycoside and carbapenem groups. As evident from table 2, higher percentage of resistance was shown against antibacterial agents from most of these classes. Notably, *Acinetobacter* sp. from our sample was found to offer remarkable resistance against cephalosporins as well as against aminoglycosides and azacytidine. In terms of percentage, highest resistance was observed against cephalexin (79%), followed by gentamicin (52%), and netilmicin (49%).

Considerably, resistance against fluoroquinolones was also notable and are in consistent with worldwide resistance data that demonstrate emergence of fluoroquinolone, cephalosporin and aminoglycoside resistant pathogens including *Acinetobacter* species (Dalhoff *et al*., 2012; Chukwu *et al*.; 2022, Padmasini *et al*., 2014 and Ayenew *et al*., 2014). In terms of molecular mechanism, extensive production of drug-inactivating enzymes by *Acinetobacter* sp. is chiefly responsible for emergence of MDR phenotype. Specifically, extended-spectrum β-lactamases (ESBLs), other β-lactamases (e.g., AmpC β-lactamase, etc.), carbapenemase and metallo-β-lactamases (MBL) are the enzymes that enable the bacteria to get rid of most of antibiotic classes and thus exhibiting MDR characteristics (Karki *et al*., 2021; Mishra *et al*., 2012; Pandey *et al*., 2021).

Table 2. Sensitivity and resistance pattern of *Acinetobacter* sp. (N = 66).

Antibiotic	Sensitive		Resistant	
	No.	%	No.	%
Ciprofloxacin	40	60.6	25	37.9
Levofloxacin	42	63.6	18	27.3
Chloramphenicol	42	63.6	19	28.8
Tetracycline	43	65.2	18	27.3
Doxycycline	43	65.2	18	27.3
Cotrimoxazole	41	62.1	23	34.8
Cephalexin	9	13.6	52	78.8
Cefoxitin	16	24.2	25	37.9
Cefuroxime	36	54.5	29	43.9
Cefixime	36	54.5	29	43.9
Cefepime	44	66.7	18	27.3
Imipenem	51	77.3	11	16.7
Meropenem	52	78.8	12	18.2
Gentamicin	28	42.4	34	51.5
Netilmicin	32	48.5	32	48.5
Amikacin	34	51.5	29	43.9
Azacytidine	31	47.0	27	40.9

Data for moderate sensitivity are not shown.

Clinical isolates of Acinetobacter sp. exhibited extended and pan-drug resistance beside MDR: Although majority of the isolated clinical samples of *Acinetobacter* sp. contained MDR (66%) pathogens, few of them also turned out to be XDR (13%) and PDR (2%) as well (Figure 1). Interestingly, we found two PDR isolates which indicated emergence of an alarming situation where control infectious diseases became more complicated probably because of being superbugs. Particularly, presence of XDR and PDR clinical isolates are not new and such isolates of *Acinetobacter* sp. have been reported in many countries across the globe (Chmielarczyk *et al*., 2018; Dimopoulos *et al*., 2015; Magiorakos *et al*., 2012; Souza *et al*., 2019 and Wong *et al*., 2017). Here, we report the clinical evidence of XDR and PDR isolates of *Acinetobacter* sp. beside MDR isolates in Bangladesh (Table 3). The percentage of such superbugs demonstrating MDR, XDR and PDR phenotype may be even more in true sense of the term. The small sample size may explain the reason of getting lower percentage. Mostly, the XDR and PDR isolates are found to be more prevalent among the intensive care unit (ICU) patients having difficult-to-treat bloodstream infections (Dimopoulos *et al*., 2015 and Souza *et al*., 2019).

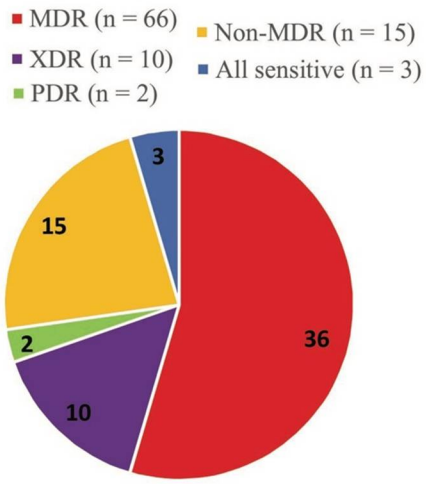

Figure 1. Distribution among the various types of resistant isolates of *Acinetobacter* sp.

Table 3. Distribution of MDR, XDR and PDR isolates of *Acinetobacter* sp. (N = 66).

Antibiotic class	Antibiotic	Non-MDR		MDR		XDR		PDR		Total R	
		No.	%	No.	%	No.	%	No.	%	No.	%
All class		24	37.5	29	45.3	8	12.5	3	4.7	64	97
Carbapenem	Imipenem R	0	0.0	4	36.4	4	36.4	3	27.3	11	17.2
	Meropenem R	0	0.0	4	33.3	5	41.7	3	25.0	12	18.8
Fluoroquinolone	Ciprofloxacin R	2	8.0	12	48.0	8	32.0	3	12.0	25	39.1
	Levofloxacin R	2	11.1	7	38.9	6	33.3	3	16.7	18	28.1
Cephalosporin (3rd gen)	Ceftriaxone R	3	13.0	10	43.5	7	30.4	3	13.0	23	35.9
	Cefixime R	4	13.8	15	51.7	7	24.1	3	10.3	29	45.3
Cephalosporin (4th gen)	Cefoxitin R	3	11.1	15	55.6	6	22.2	3	11.1	27	42.2
	Cefepime R	2	11.1	6	33.3	7	38.9	3	16.7	18	28.1
Aminoglycoside	Gentamicin R	3	8.8	20	58.8	8	23.5	3	8.8	34	53.1
	Netilmicin R	2	6.3	20	62.5	7	21.9	3	9.4	32	50.0
	Amikacin R	0	0.0	19	65.5	7	24.1	3	10.3	29	45.3

Conclusion

Antibiotic resistance is a global health problem and Bangladesh is one of the major contributors to this due to irrational use of antimicrobials and pluralistic health system. It is apparent from the study that *Acinetobacter* sp. is resistant to most of the available antimicrobial agents, and the emergence and spread of this species is an area of great concern which need urgent attention. So, it is high time we take some measures to slow down the emergence and spread of antimicrobial resistance including programs on surveillance, education and research on antimicrobial resistance and infection control. Measures should also be taken in regulating the use of antimicrobials in hospitals as well as in the community, and antimicrobial stewardship programs to prevent the further spread of these resistant bacteria.

References

Almasaudi, S. B. 2018. Acinetobacter spp. as nosocomial pathogens: Epidemiology and resistance features. *Saudi J. Biol. Sci.* **25**, 586-596.

Ayenew, Z., Tigabu, E., Syoum, E., Ebrahim, S., Assefa, D. and Tsige, E. 2021. Multidrug resistance pattern of *Acinetobacter* species isolated from clinical specimens referred to the Ethiopian Public Health Institute: 2014 to 2018 trend anaylsis. *PLoS ONE* **16**, e0250896.

Bauer, A. W., Kirby, W. M., Sherris, J. C., and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* **36**, 493-496.

Chmielarczyk, A., Pobiega, M., Ziółkowski, G., Pomorska-Wesołowska, M., Romaniszyn, D., Krawczyk, L. and Wójkowska-Mach, J. 2018. Severe infections caused by multidrug-resistant non-fermentative bacilli in southern Poland. *Adv. Clin. Exp. Med*. **27**, 401-407.

Chukwu, E. E., Awoderu, O. B., Enwuru, C. A., Afocha, E. E., Lawal, R. G., Ahmed, R. A., Olanrewaju, I., Onwuamah, C. K., Audu, R. A. and Ogunsola, F. T. 2022. High prevalence of resistance to third-generation cephalosporins detected among clinical isolates from sentinel healthcare facilities in Lagos, Nigeria. *Antimicrob. Resist. Infect. Control.* **11**, 134.

Clinical and Laboratory Standards Institute (CLSI) 2020. Performance Standards for Antimicrobial Susceptibility Testing, 30th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute. 950 West Valley Road, Suite 2500, Wayne.

Dalhoff A. 2012. Global fluoroquinolone resistance epidemiology and implications for clinical use. *Interdiscip. Perspect. Infect. Dis*. **2012**, 976273.

Davin-Regli, A., Lavigne, J. P. and Pagès, J. M. 2019. Enterobacter spp.: update on taxonomy, clinical aspects, and emerging antimicrobial resistance. *Clin. Microbiol. Rev.* **32**, e00002-19.

Diekema, D. J., Beekmann, S. E., Chapin, K. C., Morel, K. A., Munson, E. and Doern, G. V. 2003. Epidemiology and outcome of nosocomial and community-onset bloodstream infection. *J. Clin. Microbiol.* **41**, 3655-3660.

Dimopoulos, G., Koulenti, D., Tabah, A., Poulakou, G., Vesin, A., Arvaniti, K., Lathyris, D., Matthaiou, D. K., Armaganidis, A. and Timsit, J. F. 2015. Bloodstream infections in ICU with increased resistance: epidemiology and outcomes. *Minerva. Anestesiologica*. **81**, 405-18.

Gohel, K., Jojera, A., Soni, S., Gang, S., Sabnis, R. and Desai, M. 2014. Bacteriological profile and drug resistance patterns of blood culture isolates in a tertiary care nephrourology teaching institute. *Biomed. Res. Int*. **2014**, 153747.

Karki, D., Dhungel, B., Bhandari, S., Kunwar, A., Joshi, P. R., Shrestha, B., Rijal, K. R., Ghimire, P. and Banjara, M. R. 2021. Antibiotic resistance and detection of plasmid mediated colistin resistance mcr-1 gene among *Escherichia coli* and *Klebsiella pneumoniae* isolated from clinical samples. *Gut. Pathogens*. **13**, 45.

Kyriakidis, I., Vasileiou, E., Pana, Z. D. and Tragiannidis, A. 2021. *Acinetobacter baumannii* antibiotic resistance mechanisms. *Pathogens*. **10**, 373.

Lawrence, R. and Jeyakumar, E. 2013. Antimicrobial resistance: a cause for global concern. *BMC Proc.* **7**, S1.

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T. and Monnet, D. L. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect.* **18**, 268-81.

Mishra, S. K., Acharya, J., Kattel, H. P., Koirala, J., Rijal, B. P. and Pokhrel, B. M. 2012. Metallo-beta-lactamase producing gram-negative bacterial isolates. *J. Nepal Health Res. Counc*. **10**, 208-213.

Padmasini, E., Padmaraj, R. and Ramesh, S. S. 2014. High level aminoglycoside resistance and distribution of aminoglycoside resistant genes among clinical isolates of *Enterococcus* species in Chennai, India. *Sci. World J*. **2014**, 329157.

Pandey, R., Mishra, S. K. and Shrestha, A. 2021. Characterisation of ESKAPE pathogens with special reference to multidrug resistance and biofilm production in a Nepalese hospital. *Infect. Drug Resist*. **14**, 2201-2212.

Prestinaci, F., Pezzotti, P. and Pantosti, A. 2015. Antimicrobial resistance: a global multifaceted phenomenon. *Pathog. Glob. Health* **109**, 309-318.

Souza, G. L., Rocha, R. F., de A., Silveira, A. D., Nascimento, Dias Duarte de Carvalho, H., Oliveira, C. D. M., Leite, E. M. M., Silva, E. U., Giarola, L. G., Couto, B. R. G. M. and Starling, C. E. F. 2019. 2475. Incidence of multidrug-resistant, extensively drug-resistant and pandrug-resistant gram-negative bacteria in Brazilian intensive care units. *Open Forum Infect. Dis.* **6**, S857.

Towner, K., J. 2009. Acinetobacter: an old friend, but a new enemy. *J. Hosp. Infect.* **73**, 355-363.

Towner, K., J. 1997. Clinical importance and antibiotic resistance of *Acinetobacter* spp. Proceedings of a symposium held on 4-5 November 1996 at Eilat, Israel. *J. Med. Microbiol*. **46**, 721-746.

Wong, D., Nielsen, T. B., Bonomo, R. A., Pantapalangkoor, P., Luna, B. and Spellberg, B. 2017. Clinical and pathophysiological overview of *Acinetobacter* infections: A century of challenges. *Clin. Microbiol. Rev*. **30**, 409-447.