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ANTIMICROBIAL RESISTANCE PATTERNS AMONG *ACINETOBACTER BAUMANNII* ISOLATED FROM THONG NHAT DONG NAI GENERAL HOSPITAL

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ABSTRACT : *Acinetobacter baumannii (Ab) is a troublesome and increasingly problematic healthcare-associated pathogen, especially in critical care unit (ICU) and cardiovascular internal medicine (CIM). This organism has a capacity for long-term survival in the hospital environment. This study aimed to investigate the drug resistance patterns of Ab strains isolated from Thongnhat Dongnai General Hospital and the relationships between Ab isolations with clinical wards and year of patients. The antibiotic susceptibility of 279 Ab isolates for aminoglycosides, fluro-quinolons, cephalosporins, carbapenems, colistin and bactrim was determined using Kirby-Bauer disk diffusion method. The minimum inhibitor concentration (MIC) of 146 Ab isolates for Meropenem was determined using E-test method according to CLSI guide-line. A total 279 A. baumannii strains out of 1,976 positive isolates were collected from various specimens during study period. Among Ab-positive specimens, the most isolated specimen was sputum (26.6%, χ^2 =161.705 p<0.000), two of the most isolated clinical wards were ICU (22.19%, χ^2 =80.854, p<0.000) and Cardiovascular Internal Medicine (CIM) (27.4%, χ^2 =27.9979, p<0.000), the most isolated age group was from 76 to 99 (22.95%, χ^2 =27.9979, p<0.000). Among 279 Ab isolated, resistance from 53.16% – 63.52% to aminoglycosides, 23.6% – 68.58% to fluroquinolons, 59.61% to 97.93% to cephalosporins, 60.27% to 80.7% to carbapenem, 10.53% to 66.48% to antibiotic combinations, 0.75% to colistin and 61.71% to bactrim. Among 146 multidrug-resistant Ab, 53.42% $MIC_{meropenem} \geq 32$ µg/ml and only 18.49% strains were susceptible to Meropenem. Due to the high antimicrobial resistance to two clinical wards (ICU and CIM) and carbapenems by disk agar diffusion test and E-test; we must focus on both a wiser use of antimicrobials and the prevention of infection. Continuous monitoring of antimicrobial susceptibility and strict adherence to infection prevention guidelines are essential to eliminate major outbreaks in the future.*

KEYWORDS: *Acinetobacter baumannii (Ab), carbapenem, MIC, ICU, CIM.*

I. INTRODUCTION

Acinetobacter baumannii is an aerobic, non-lactose fermenting, oxidase - negative, Gram-negative coccobacillus that is most commonly found associated with healthcare environments. Although initially considered of low pathogenic potential in healthy individuals, *Ab* is now largely considered as an important pathogen implicated in nosocomial infections. *Ab* can survive on moist and dry surfaces and may be present in foodstuffs and on healthy skin. These factors, together with both intrinsic and acquired antibiotic resistance, account for the success of *Ab* as a significant cause of outbreaks and endemic spread of resistant clones throughout the world. Significant cost, morbidity and mortality have been reported with outbreaks and even criminal charges have been directed against hospitals where outbreaks have occurred [1]. It is probably now accounts for 2-10% of Gram-negative bacterial infections in ICUs in Europe and the United States [2], up to more than 16% in Thongnhat Dongnai General Hospital, Viet Nam [4]. The epidemic potential and the clinical severity of *Ab* infections are primarily related to the ability to survive and spread within hospital environment and to develop resistance to a variety of antimicrobial agents, including broad-spectrum beta-lactams, fluoroquinolones, aminoglycosides, and carbapenems [3]. Despite the increasing significance and frequency of multidrug-resistant *Ab* infections, many clinicians still lack an appreciation of the importance of these organisms in hospitals, in part of their confused taxonomic status [5]. The aim of the study was to determine the drug resistance patterns of *Ab* strains isolated from different clinical wards using Kirby-Bauer disk diffusion method, E-test and their relationships with risk factors at Thongnhat Dongnai General Hospital, Vietnam.

II. MATERIALS AND METHODS

2.1 Bacterial isolates

All *Ab* clinical isolates identified in the Thongnhat Dongnai General Hospital (Southern of Vietnam) were selected. Initially conventional biochemical tests such as Gram stain, catalase, and oxidase tests were used [6]. Then, *Acinetobacter* spp. were characterized by phenotypic method by using API 20NE (bio-Mérieux, France) for the identification at the species level. Two hundred and seventy-nine non-duplicate *Ab* isolates recovered from routine cultures performed in the microbiology laboratory from wounds and abscesses swabs, urine, blood culture, and others, derived from different clinical wards.

2.2 Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined by both the Kirby-Bauer disk diffusion method [7] and E-test method for minimum inhibitory concentration (MIC) determination [8]. *Acinetobacter baumannii* ATCC 19606 was included as control. The tests were read after overnight incubation and the following antibiotics were used: Amino-glycosides (Amikacin, Gentamicin, Netilmicin), Fluro-quinolons (Ciprofloxacin, Levofloxacin), Doxycycline, Cephalosporins (Cefpodoxim, cefuroxim, cefotaxim, ceftazidime, cefepime), Carbapenems (Imipenem and Meropenem), antibiotics combination (Amoxicillin + Clavunalic acid, Ampicillin + Sulbactam, Ceftazidime + Clavunalic acid, Ticarcillin + Clavunalic acid, Piperacillin + Clavunalic acid) and the others (Colistin and Bactrim). Only the Meropenem was used for MIC detection according to CLSI guide-line.

2.3 Statistics

The descriptive cross-sectional study is analyzed by the Stata 8.0 program [9]. The data was collected by Epi-data 3.1 software and use the Chi-square test to compare two unpaired groups. This difference is statistically significant with $p<0.05$.

III. RESULTS

During the study, 279 *Ab* isolates were isolated from patients, representing 279/1,976 (14.12%) of total positive samples. There were 40.78% females and 59.22% males. The majority of strains were derived from ICU (n=174, 22.19%) and CIM (n=20, 27.4%) compared with the others (n=85, 50.1%), with χ^2=80.854; $p<0.000$ (**Table 1**). The patients included in the study their ages ranged between 14 – 99 years, with a mean of 57.62 years and the statistics showed that the higher the patient's age, the percentage of *Ab*-positive specimens cultured higher, with χ^2=27.9979; $p<0.000$ (**Table 1**). The body sites and fluids from which the organism was recovered included sputum (26.6% of the isolates), wound and abscess swabs (4.39%), blood (3.43%), body secretions (8.11%) and urine (1.43%), with χ^2 = 161.705 and $p<0.000$.

Table 1. The percentage of *Ab*-positive specimens in the clinical wards, %[A]; the age group, %[B]

No.[A]	The clinical wards[A]	*Ab*[A]	No.[B]	The age group[B]	*Ab*[B]
1	ICU	22.19	1	14 - 45	11.62
2	Tropical disease	4.71	2	46 - 60	10.46
3	Orthopedics	2.30	3	61 - 75	17.27
4	General Internal	17.1	4	76 - 99	22.95
5	Cardiovascular Internal	27.4	*Pearson chi2(3) = 27.9979; Pr = 0.000*		
6	Obstetrics	4.17			
7	General Surgery	4.17			
8	Internal Medicine Kidney	5.41			
9	Neurological Surgery	10.26			

Ab ranked the first most frequently isolated microorganism isolated from patients in ICU, followed by *Pseudomonas aeruginosa, Klebsiella pneumoniae, E. coli, Candida* and they ranked the second one in CIM preceded by Candida. The antimicrobial resistance is shown in **Table 2a**, during one and a-half year of the study, all *Ab* isolates were highly resistant to Aminoglycosides (53.16% to 65.52%), Fluroquinolons (56.45% - 68.58%), Doxycycline within 23.6%, Bactrim (61.71%), the third generation cephalosporins (73.43% to 97.93%), the 4th cephalosporin (resist up to 59.61% to cefepime). A worrying situation is the high rates of resistance also demonstrated to Carbapenems (60.27% - 76.92%) and most of antibiotic combinations (resistance more than 50%, except of Ampicillin plus Sulbactam within 10.53%).

Table 2a. Antimicrobial susceptibility results of *Acinetobacter baumannii* isolates

Antibiotics	% Resistance	% Sensitivity	Antibiotics	% Resistance	% Sensitivity
Amikacin	58.85	37.04	Cefpodoxim	97.93	00.00
Gentamicin	65.52	33.10	Cefatadizime	73.43	19.32
Netilmycin	53.16	46.32	Cefepime	59.61	34.12
Ciprofloxacin	68.58	30.97	Imipenem	62.39	36.24
Levofloxacin	56.45	43.55	Meropenem	60.27	37.50
Doxycycline	**23.60**	**74.80**	**AMP + sulb**	**10.53**	**78.36**
Colistin	**0.75**	**98.50**	Ceftazidime/ A. Clavulanic	57.14	39.29
Cefpodoxim	97.93	00.00	Ticarcillin/ A. Clavulanic	66.48	30.11
Ceftazidime	73.43	19.32	Piperacillin/ Tazobactam	58.37	36.48

According to **Table 2b**, of *Ab* isolates in this study, ICU isolates exhibited significantly higher level of resistance to Imipenem (83.77%) and Meropenem (83.97%) compared with non-ICU strains (35.14% and 32.88% respectively) (p<0.01). In addition, this study revealed that almost ICU isolates were more resistant than non-ICU strains (almost p<0.001) but Cardiovascular Internal Medicine isolates were not (p >0.05, unpublished data).

Table 2b. Relationship between Clinical Department Isolates and antimicrobial resistance

Antimicrobial agents	ICU		Non – ICU (*Except CIM*)		Person chi^2	*p* value
	n	%	n	%		
Amikacin	141	82.94	24	32.43	60.0748	0.000
Netilmicin	106	73.61	14	23.73	43.0868	0.000
Ceftazidime	138	94.52	33	53.23	50.7457	0.000
Cefpodoxime	117	99.15	61	95.31	2.8466	0.092
Ceftriaxon	148	100	66	92.96	10.6661	0.001
Cefotaxim	120	96.00	63	91.30	1.8328	0.176
Cefepime	126	84.00	35	41.18	46.1196	0.000
Imipenem	**129**	**83.77**	**26**	**35.14**	**54.3076**	**0.000**
Meropenem	**131**	**83.97**	**24**	**32.88**	**59.3637**	**0.000**
Doxycycline	61	34.66	12	14.63	11.0559	0.001
Ciprofloxacin	140	86.96	32	43.84	47.9535	0.000
Ticarcillin/A.Clavulanic	112	86.15	17	34.69	46.8136	0.000
Piperacillin/Tazobactam	130	82.28	28	36.36	49.5421	0.000
Bactrim	123	80.92	31	42.47	33.7662	0.000

Among 146 multidrug-resistant *Ab*, 53.42% strains (n=78) were MIC of Meropenem more than 32 µg/ml (60.94% strains were resistant to Meropenem) and only 36.96% strains (n=54) were susceptible to Meropenem (MIC ≤4 µg/ml, CLSI 2013) (**Table 3**).

Table 3. MICs of Meropenem in the tested *Acinetobacter baumannii*

Order No.	MIC of Meropenem, µg/ml	*Percentage, %*	Freq.	Susceptibility Phenotype
1	≤ 4	36.96	54	Sensitivity
2	>4 to 8	2.05	2	Intermediate
3	> 8 to 24	7.52	11	Resistance
4	**≥ 32**	**53.42**	**78**	**Resistance**

IV. DICUSSION

Within a few decades ago *Ab* rapidly developed resistance to a wide variety of antibiotics that emerged in many parts of the world, mostly by acquisition of gene clusters carried by plasmids, transposons, integrons, and resistance islands within the genome [10-14]. This phenomenon led to the emergence of increasingly multidrug resistance in this species [5]. Moreover, resistance patterns among nosocomial bacterial pathogens may vary widely from country to country at any given point and within the same country over time. *Ab* ranked the first most frequently isolated microorganism isolated from patients in ICU, followed by *Pseudomonas aeruginosa, Klebsiella pneumoniae, E. coli, Candida* and they ranked the second one in CIM preceded by Candida. Ampicillin/sulbactam, Colistin (Polymycin family) and Doxycycline remained the antimicrobial most active antibiotic against *Ab* isolates compared with other antibiotics. Sulbactam, a beta-lactamase inhibitor, also exhibits intrinsic activity against *Acinetobacter* spp., including carbapenem-resistant strains, and therefore represents an alternative to treatment with polymycin (such as Colistin) [15]. ICU isolates exhibited significantly

higher level of resistance to imipenem (83.77%) and meropenem (83.97%) compared with non-ICU strains (35.14% and 32.88% respectively), within $p<0.001$. *Ab* and could disseminate in the ICU, probably after contamination of the hospital environment and by nosocomial transmission [16, 17, 18-21]. A high resistance rate to imipenem and meropenem in *Acinetobacter* spp. isolates may lead to extensive use of Colistin. In our study, among 146 Multidrug Resistance-*Ab* of 279 strains, 60.94% strains were resistant to Meropenem using E-test method. This result was lower than a report from 16 other hospitals in Vietnam that revealed resistance rates of 6.5% of MIC=16 µg/ml, 2.4% of MIC = 24 µg/ml, 63.7% of MIC = 32 µg/ml and 27.4% of MIC >32 µg/ml for meropenem, using the same method [22]. Totally, it seems that the resistance of *Ab* is constantly changing and the consideration of this change is necessary in various countries. It seems that a surveillance of nosocomial pathogens for resistograms is needed for every country and/or even for every hospital in order to guide appropriate selection for empiric therapy.

V. CONCLUSION

Due to the high possibility of transmission of the antibiotic resistance through a variety of transmissible elements include plasmid and presence of many asymptomatic colonizers has raised the importance of active surveillance to identify potential colonizers and reservoirs of the microorganism combined with implementation of infection control measures, including strict hand hygiene, appear to be effective in controlling such outbreaks of multi- drug resistant bacteria in clinics and hospitals. Furthermore, such measures may reduce infection, mortality rates, and length of hospitalization and associated costs.

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