A STUDY OF ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF CLINICAL ISOLATES OF ACINETOBACTER SPECIES AT A TERTIARY CARE HOSPITAL IN CENTRAL INDIA

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ABSTRACT

BACKGROUND

Acinetobacter have been implicated in a variety of nosocomial infections including ventilator associated pneumonia, bacteraemia, urinary tract infection, secondary meningitis and in patients confined to hospital intensive care units. Multidrug resistant Acinetobacter species have limited the options for effective treatment.

The present study was undertaken to isolate and identify the multidrug resistant (MDR) and pan drug resistant (PDR) Acinetobacter species and also to determine minimum inhibitory concentration of tigecycline using agar dilution method.

MATERIALS AND METHODS

This is a prospective study conducted over a period of 22 months (January 2014 to October 2015) in a tertiary care hospital. Ninety-six non-duplicate clinical isolates of Acinetobacter species were processed for species identification by standard Microbiological procedures. Antimicrobial susceptibility of these isolates was performed by Kirby-Bauer disc diffusion method and minimum inhibitory concentration of Tigecycline was tested using agar dilution method.

RESULTS

A. baumannii was the most common species isolated accounting for 82.29% followed by *A. calcoaceticus* (10.41%), *A. haemolyticus* (3.12%), *A. lwoffii* (3.12%) and *A. junii* (1.04%). Isolates from ICU specimens is significantly higher than ward (p value < 0.05). 89 (92.71%) out of 96 Acinetobacter isolates were multidrug resistant and there was no isolate which was found to be PDR. By MIC agar dilution methods for tigecycline, 82 (85.41%) isolates were found to be sensitive to tigecycline.

CONCLUSION

Increasing use of antibiotics in hospitals is leading towards antibiotic resistance, which in turn exerts selective pressure on Acinetobacter in hospital environment. Continued surveillance combined with preventive measures remains absolutely essential in efforts to prevent or limit spread of Acinetobacter and resulting infections.

KEYWORDS

Acinetobacter, Multidrug Resistant, Pan Drug Resistant, Tigecycline, Minimum Inhibitory Concentration.

HOW TO CITE THIS ARTICLE: Kusalkar MV, Seema AM, Vyankatesh KJ. A study of antimicrobial susceptibility pattern of clinical isolates of Acinetobacter species at a tertiary care hospital in central India. J. Evolution Med. Dent. Sci. 2018;7(01):53-57, DOI: 10.14260/jemds/2018/14

BACKGROUND

Among myriad living organisms, Acinetobacter species have quickly gone from being an unknown and unnamed organism to becoming amongst the most dangerous pathogenic organism of present times.⁽¹⁾ Over the last 30 years, gram negative bacilli have proved to be a particular problem in causing human infection. After the therapeutic introduction of newer broad-spectrum antibiotics in hospitals, an increase in the incidence of resistance is seen in Acinetobacter species.⁽²⁾ They have been implicated in a variety of nosocomial infections including bacteraemia, urinary tract infection and secondary meningitis, but their role as agents of nosocomial pneumonia, particularly ventilator associated pneumonia in patients confined to hospital intensive care

units is important.⁽³⁾ In addition to infection among hospitalised patients, community-acquired Acinetobacter infections is increasingly reported. The common and important representative of the genus is *Acinetobacter baumannii* and other species such as *Acinetobacter lwoffii*, *Acinetobacter johnsonii* and *Acinetobacter haemolyticus* are less frequently isolated from patients.⁽⁴⁾

Predisposing factors for Acinetobacter infections include presence of prosthesis, endotracheal intubation, intravenous catheters and prior antibiotic therapy in a seriously ill patient in hospital.⁽⁵⁾ Such infections are often extremely difficult for the clinician to treat, because of increasing resistance of these bacteria to the major groups of antibiotics. Of particular concern, *A. baumannii* strains exhibiting resistance to carbapenems which were previously considered the treatment of choice for infected patients have dramatically increased worldwide, leaving a few antibacterial choices. Tigecycline, a broad-spectrum modified minocycline derivative, is considered as a last resort drug against multidrug-resistant *A. baumannii*. Though, resistance to tigecycline has emerged and is growing notably following increasing tigecycline usage.⁽⁶⁾ For this study, Multidrug resistant (MDR) Acinetobacter spp. are defined as those isolates resistant to more than three classes of antibiotics.

'Financial or Other Competing Interest': None.

Submission 05-11-2017, Peer Review 17-12-2017,

Acceptance 22-12-2017, Published 01-01-2018.

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DOI: 10.14260/jemds/2018/14

Pan drug resistant (PDR) *Acinetobacter* spp. are those resistant to all the commonly used antibiotics.⁽⁷⁾

MATERIALS AND METHODS

Study Design

Descriptive type of study.

Sample Collection

The present prospective study was conducted in the Department of Microbiology at a tertiary level teaching health care facility over a period of 22 months (January 2014 to October 2015). Ninety six non-duplicate *Acinetobacter* isolates recovered from the urine, pus, blood, respiratory samples such as endotracheal aspirates, bronchoalveolar lavage (BAL), CSF and other body fluids were included in the study.

Isolation and Identification of *Acinetobacter* Species

For the isolation of *Acinetobacter* spp., the clinical samples were inoculated onto blood agar and MacConkey agar. After overnight incubation at 37°C, the suspected colonies were further processed by standard microbiological methods.⁽⁸⁾ Speciation of the *Acinetobacter* isolates was done as per the biochemical tests described in Table 1.⁽⁹⁾

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing of all the 96 *Acinetobacter* isolates was carried out by Kirby-Bauer disc diffusion method on Mueller-Hinton agar medium and results were interpreted as per the Clinical and Laboratory Standards Institute guidelines.⁽¹⁰⁾

Antimicrobial discs used in the study were procured from Hi-Media Laboratories, Mumbai, India. Following antibiotic discs were put up with the concentration of the compound mentioned in the parenthesis: ceftazidime (30 µg), imipenem (10 µg), meropenem (10 µg), gentamicin (10 µg), tobramycin (10 µg), ampicillin-sulbactam (10/10 µg), ciprofloxacin (5 µg), piperacillin (100 µg), piperacillin-tazobactam (100/10 µg), ticarcillin-clavulanic acid (75/10 µg), cefepime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), amikacin (30 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), Polymyxin B and tigecycline (15 µg).

Testing of Metallo-β-Lactamase (MBL) Production

Imipenem resistant isolates were further screened for metallo-beta-lactamase production by combination disc test.⁽¹¹⁾

Minimum Inhibitory Concentration (MIC)

MIC for all *Acinetobacter* isolates was determined for tigecycline (TG) by agar dilution method,⁽¹²⁾ tigecycline powder procured from Hi-Media Laboratories.

Statistical Analysis

Percentage were used to describe the proportion. Chi-square test was used as the test of significance for categorical variables. 'P' value less than 0.05 was considered to be statistically significant.

RESULTS

A total of 96 non-duplicate, non-consecutive *Acinetobacter* isolates were processed for species identification,

antimicrobial susceptibility testing and to know the MDR and PDR pattern of these isolates. A total of 5681 clinical samples were processed, of which 96 (1.69%) strains of *Acinetobacter* were isolated. Table 2 shows that 71 (1.29%) *Acinetobacter* strains were isolated from 5480 specimens of ward and 25 (12.44%) isolates were from 201 ICU specimens. Percentage of Isolates from ICU specimens is higher than ward. *A. baumannii* was the most common species isolated accounting for 82.29% followed by *A. calcoaceticus* (10.41%), *A. haemolyticus* (3.12%), *A. lwoffii* (3.12%) and *A. junii* (1.04%). The percentage of isolation of *A. baumannii* was higher (82.29%) as compared to other species (17.71%). The pattern of distribution of *Acinetobacter* species in various infections is reflected in Table 3. The antimicrobial susceptibility pattern of *Acinetobacter* is shown in Table 4. Table 4 shows 100% sensitivity to Polymyxin B, 85.42% to Tigecycline, 75% to ampicillin-sulbactam, 66.66% sensitive to Imipenem and 62.50% to meropenem. Maximum resistance was seen to ciprofloxacin (97.91%) followed by piperacillin 95.83%, cefepime (90.60%), cotrimoxazole (85.42%) and cefotaxime (81.25%). Imipenem resistance was seen in 32 (33.33%) isolates. 89 (92.71%) out of 96 *Acinetobacter* isolates were multidrug resistant and 7 (7.29%) were non-MDR. None of the isolates recovered was resistant to Polymyxin B. Thus, there was no isolate, which was found to be PDR. *Acinetobacter* isolates recovered from ICU patients were found to be more drug resistant than those isolated from ward and outdoor patients. 32 imipenem resistant isolates were tested for MBL production. Out of the total 32 imipenem resistant *Acinetobacter* isolates, 28 (87.5%) were MBL producer and all of them were *A. baumannii* strains. By MIC agar dilution and disc diffusion methods for tigecycline, 82 (85.41%) isolates were found to be sensitive to tigecycline. Disc diffusion showed 14 isolates as resistant, of which 9 turned out to be intermediate and only 5 as resistant by MIC.

Acinetobacter Species	Growth at		Haemolysis	Gelatin Hydrolysis	of Glucose	Arginine Dihydrolase
	37° C	44° C				
<i>A. baumannii</i>	+	+	-	-	+	+
<i>A. calcoaceticus</i>	+	-	-	-	+	+
<i>A. haemolyticus</i>	+	-	+	+	V	+
<i>A. lwoffii</i>	+	-	-	-	-	-
<i>A. junii</i>	+	-	-	-	-	+
<i>A. johnsonii</i>	-	-	-	-	-	V

Table 1. *Acinetobacter* Species Identification

Wards	No. of Specimens Processed	Acinetobacter Isolates	
		Number	Percentage
Wards	5480	71	1.29
ICU	201	25	12.44
Total	5681	96	1.69

Table 2. Number of *Acinetobacter* Isolates

(p value < 0.05)

Infection	<i>A. baumannii</i> (%)	<i>A. calcoaceticus</i> (%)	<i>A. haemolyticus</i> (%)	<i>A. lwoffii</i> (%)	<i>A. junii</i> (%)	Total (n)
Skin and soft tissue infection	38(86.36)	5(11.36)	1(2.28)		-	44
Septicaemia	14(63.64)	2(9.09)	2(9.09)	3(13.63)	1(4.54)	22
Pneumonia	13(86.67)	2(13.33)				15
UTI	6(85.72)	1(14.28)				7
Ascites	5	-				5
Pleural effusion	2	-				2
Meningitis	1	-				1
Total	79	10	3	3	1	96

Table 3. The Pattern of Distribution of Acinetobacter Species in Various Infections

Group	Antimicrobial Agent	Resistant Isolates (n= 96)	
		No.	%
Group A	Ceftazidime (CAZ)	72	75.00
	Imipenem (IMP)	32	33.33
	Meropenem (MRP)	36	37.50
	Gentamycin (G)	76	79.16
	Tobramycin (TOB)	78	81.25
	Ampicillin sulbactam (AS)	24	25.00
	Ciprofloxacin (CIP)	94	97.91
Group B	Piperacillin (PIP)	92	95.83
	Piperacillin-Tazobactam (PIT)	72	75.00
	Ticarcillin-Clavulanic acid (TCC)	78	81.25
	Cefepime (CPM)	87	90.62
	Cefotaxime (CTX)	78	81.25
	Ceftriaxone (CTR)	76	79.16
	Amikacin (AK)	72	75.00
Group O	Tetracycline (T)	80	83.33
	Trimethoprim-Sulfamethoxazole (COT)	82	85.42
Group O	Polymyxin (PB)	0	-
Other	Tigecycline (TGC)	14	14.58

Table 4. Antimicrobial Sensitivity Pattern of Acinetobacter Isolates (n= 96)

Wards	Total No. of Isolates	MDR Isolates	
		Number	Percentage
ICU	25	25	100.00
Surgery	34	31	91.18
Obstetrics and Gynaecology	11	10	90.90
Medicine	15	14	93.33
Paediatrics	8	7	87.50
Orthopaedics	3	2	66.66
Total	96	89	92.70

Table 5. Distribution of Multiple Drug Resistant (MDR) Isolates in various Hospital Wards

DISCUSSION

Acinetobacter species are contributing profoundly to the burden of modern medicine, where the term 'burden' refers to the sum of clinical and economic load.⁽¹³⁾ The rate of isolation of Acinetobacter species from our study was 1.6%. Various other studies have reported the rate of isolation varying from 1.54% to 20.1% (Jaggi et al⁽¹⁴⁾ 2012), Dash et al (2013) and Behera et al 2011.⁽¹⁵⁾ This variation can be attributed to the varying prevalence rates of different Acinetobacter species in the hospital environment and the community in different geographical areas. However, *A. baumannii* is seldom recognised as a true environmental

organism. As other study groups (Idmir et al,⁽¹⁶⁾ Mindoli et al,⁽¹⁷⁾ Tripathi et al,⁽¹⁸⁾ *A. baumannii* was the most common species isolated accounting for 82.29% followed by *A. calcoaceticus* (10.41%), *A. haemolyticus* (3.12%), *A. lwoffii* (3.12%) and *A. junii* (1.04%). In this study, isolation of *A. baumannii* was significantly higher (82.29%) as compared to other species (17.71%). This was statistically significant with 'p' value less than 0.001. Most common infection caused by Acinetobacter was skin and soft tissue infection (45.83%) followed by septicaemia (22.91%) and pneumonia (15.63%). In the current study, among the different risk factors diabetes mellitus was the most common risk factor (41.67%) associated with all types of Acinetobacter infections studied. This is concurrent with other studies (Tripathi et al).¹⁸ Along with prolonged hospital stay intravenous catheter (63.64%) was the commonest risk factor in septicaemia, mechanical ventilation (53.33%) was the commonest risk factor in pneumonia and urinary catheter (85.71%) was the commonest risk factor in UTI. This is concurrent with other studies. In present study, Acinetobacter strains showed maximum sensitivity to polymyxin B (100%) followed by tigecycline (85.42%), ampicillin-sulbactam (75%), imipenem (66.66%) and meropenem (62.50%). Maximum resistance was noted to ciprofloxacin (97.91%). Resistance to piperacillin, cotrimoxazole and tetracycline was 95.83%, 85.42% and 83.33 respectively. Among cephalosporins, out of 96 isolates 90.62% showed resistance to cefepime, 81.25% to cefotaxime, 79.16% to ceftriaxone and 75% to ceftazidime in our study. Taneja et al⁽¹⁹⁾ in 2012 showed that the resistance of Acinetobacter to gentamicin, amikacin and ciprofloxacin was 79.5%, 73.2% and 72.8% respectively. Shareek et al in 2012 reported that only 25% of the strains were sensitive to carbapenems, 10% - 15% of the strains were sensitive to β -lactams and 20% - 28% of the strains were sensitive to amikacin, ciprofloxacin and cotrimoxazole.⁽²⁰⁾ Other studies have also shown similar results for different antimicrobial agents.^(21,22) Resistance to polymyxins has also been reported by many authors,^(20,23) but in our study we did not notice any resistance to these antibiotics. In the current study, out of 96 isolates 89 (92.71%) were multidrug resistant (MDR). All the 25 isolates from ICU were MDR. Dash et al (2013) recorded MDR in 54.7% isolates. Badave et al⁽²⁴⁾ found 90.3% isolates to be MDR. Taneja et al⁽¹⁹⁾ also reported 41.5% of the Acinetobacter isolates as MDR.⁽²⁰⁾ In our study of the 32 imipenem resistant Acinetobacter strains, 28 Acinetobacter were MBL producers, all of which were *A. baumannii*.

Goel et al⁽²⁵⁾ found 48.72% MBL producing strains in 2013, Kaur et al⁽²⁶⁾ in 2014 found MBL activity in 80.3% of isolates and Gupta et al⁽²⁷⁾ found 14.4% MBL producing strains in 2015. By agar dilution method (Table 6) 82

(85.41%) isolates were found to be sensitive, 9 (9.38%) isolates were found to be intermediate resistant and 5(5.21%) isolates were resistant. No isolate grew at MIC 16 microgram/mL. A study⁽²⁸⁾ in Singapore in 2007 *Acinetobacter* showed 9% resistance, 20% intermediate resistant and 71% sensitive to tigecycline by agar dilution method. In 2010, Manoharan et al⁽²⁹⁾ performed tigecycline MIC by agar dilution and E strip and found that more than 90% isolates of *Acinetobacter* were having MIC < 8 mcg/mL.

CONCLUSION

Acinetobacter baumannii has already been notified a “red alert” pathogen by the Infectious Disease Society of America. *Acinetobacter* is a leading cause of wide spectrum of infections, especially in critically ill patients in ICU patients. The high prevalence of multidrug resistant mechanism makes them the “superbug” of the hospital environment. Thus, there is urgent need to control the spread of this pathogen in various hospital areas. MIC detects intermediate resistant isolates, for which treatment is possible by increasing the concentration/ dose of drug. Thus, in case of MDR strains where treatment options are limited, carbapenems and tigecycline sensitivity testing should be done by MIC whenever possible. MBL production has been shown by *Acinetobacter* strains, thus laboratories are advised to set up MBL detection testing to identify and prevent the spread of MBL strains. Continued surveillance of prevalent organisms in ICUs and other wards of hospital combined with preventive measures (e.g. isolation precautions, hand disinfection, efficient sterilisation of instruments) remains absolutely essential in efforts to prevent or limit spread of *Acinetobacter* and resulting infection.

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