

Evaluation of multiple antibiotic resistance (MAR) index and Doxycycline susceptibility of *Acinetobacter* species among inpatients

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Abstract

Background: *Acinetobacter* contributes to increased morbidity and mortality with its strong propensity to colonize and disseminate among humans and environmental sources coupled with its ability to develop resistance to antimicrobial agents.

Materials and Methods: Clinical isolates of *Acinetobacter* recovered from routine samples of inpatients were analyzed retrospectively along with their antibiogram to evaluate in vitro activity of Doxycycline. Multiple antibiotic resistance index was calculated and interpreted.

Results: Out of 93 isolates of *Acinetobacter species* recovered, predominant were from urine 47(50.54%) and blood 27(29.03%) samples. MDR isolates were 57(61.29%). Overall antimicrobial susceptibility pattern revealed best spectrum of activity with Imipenem (75.27%), Meropenem (68.82%) and Doxycycline (68.82%) whereas in MDR isolates Doxycycline exhibited highest sensitivity (66.67%) followed by Imipenem (61.40%) and Meropenem (52.63%). MAR indexes for different isolates revealed 71 (76.34%) with MAR index greater than 0.2 and 22 (23.66%) less than 0.2. However, three isolates had shown MAR index of 01 (i.e. resistant to all the antimicrobials tested), out of which two were recovered from intensive care unit and one from general surgery ward. Twenty-six MDR patterns were observed with nine antimicrobials tested. Resistance to COT, CIP, GEN, AK, A/S, CPM, IMP, MRP (R8) was most frequently observed pattern in 8(14.04%) of MDR isolates.

Conclusion: Doxycycline has exhibited efficacy against MDR *Acinetobacter*, which can be considered as an alternative therapy to down regulate selective pressure on carbapenems. To confront the immediate threat of *Acinetobacter* infections, a working antibiotic strategy should be addressed and stringent infection control practices are needed to prevent the spread of multi drug resistant isolates in the hospital.

Keywords: *Acinetobacter* species, Doxycycline, MAR index

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Introduction

Acinetobacter species are non-fermenting aerobic gram-negative bacteria that have become important nosocomial pathogens. Previously, this organism was considered to have low virulence, generally more capable of colonizing than infecting. However, it has emerged as an invasive and life-threatening pathogen, especially in critically ill patients^[1]. Interest in *Acinetobacter spp.* has been growing for the past 30 years. One of the main reasons for the present increased interest in this genus is the emergence of multiresistant strains, some of which are pan-resistant to antibiotics, which suddenly cause an outbreak of infection involving several patients in a clinical unit^[2]. The most common and serious MDR pathogens have been encompassed within the acronym “ESKAPE,” standing for *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*,

Pseudomonas aeruginosa and *Enterobacter spp*^[3]. During the mid-1990s, carbapenem resistance emerged worldwide in the genus *Acinetobacter*. Coupled with this was a concomitant emergence of *Acinetobacter spp.* as a nosocomial pathogen, raising concerns over treatment options for this organism^[4]. *Acinetobacter* being ubiquitous in nature, its ability to survive in varying temperatures, pH conditions and on dry, moist surfaces helps in transmission of this organism in hospital settings. Carbapenems which were the mainstay of therapy are no longer effective in controlling the infections caused by members of this genus^[5]. *A. baumannii* infection is now a common worldwide problem that can lead to increased morbidity and prolonged hospital stays^[1]. The risk of colonization and subsequent infection are associated with factors such as the presence of underlying severe illnesses, long term hospitalization, stays in intensive care units (ICUs), selective antimicrobial pressure, and invasive interventions such as use of mechanical ventilation or catheters. The nosocomial infections caused by *Acinetobacter* include pneumonia, septicemia, wound sepsis, urinary tract infection, endocarditis, and meningitis^[6]. The rapid emergence of multi- and pan drug-resistant strains of *Acinetobacter* highlights the organism’s ability to quickly acclimatize to selective changes in environmental pressures. The up regulation

of the organism's innate resistance mechanisms coupled with the acquisition of foreign determinants have played a crucial role in the express route the organism has taken to becoming a multidrug-resistant pathogen^[7]. The alternative therapeutic strategies for MDR *Acinetobacter baumannii* includes the use of Rifampicin, Travofloxacin, Doxycycline, Minocycline or Tigecycline with sulbactam^[8]. Multiple antibiotic resistance (MAR) indexing has been shown to be a cost effective and valid method of bacteria source tracking. Multiple antibiotic resistance index is calculated as the ratio of number of antibiotics to which organism is resistant to total number of antibiotics to which organism is exposed. MAR index values greater than 0.2 indicate high risk source of contamination where antibiotics are often used^[9]. The global problem of antimicrobial resistance is particularly pressing in developing countries, where the infectious disease burden is high and cost constraints prevent the widespread application of newer, more expensive agents^[10]. This study was carried out to evaluate MAR indices of clinical isolates of *Acinetobacter species* to track the source of origin and their susceptibility to Doxycycline, as antibiotic resistance is increasingly compromising the outcome of *Acinetobacter* infections and limiting therapeutic options among inpatients.

Materials and Methods

Study area and sample size

The study was conducted at BPS, GMC for Women Khanpur Kalan in North West region of India. It is a tertiary care health institution catering to wide rural population of Haryana and neighboring states. A total of 93 isolates belonging to *Acinetobacter spp* were included in the study. These isolates were derived from blood, urine, pleural fluid, sputum, bronchoalveolar lavage fluid, purulent wounds, intravenous catheter tips, endotracheal tube tip and tracheal aspirates collected from patients admitted in various clinical wards and intensive care units. The *Acinetobacter spp* isolates were analyzed retrospectively by retrieving data from bacteriology section of Microbiology department.

Identification and antimicrobial susceptibility testing

Various samples received in bacteriology section for aerobic bacterial cultures were inoculated on blood agar and MacConkey agar and incubated at 37°C as per standard operative guidelines. After 24 hours of incubation, non-lactose fermenting gram negative cocco-bacilli which were Catalase positive, oxidase negative, and produced an alkaline reaction on Triple Sugar Iron Agar were provisionally considered to be NFGNB. Further identification and confirmation of *Acinetobacter spp* was done using bio-chemical tests as per standard operating procedures which included hanging drop preparation, utilization of 10% glucose with Oxidation-Fermentation medium and citrate

utilization test. Isolates of *Acinetobacter spp* were differentiated from other oxidase negative, non motile, non fermenting bacilli like *Bordetella holmesii* and CDC group1 by nitrate reduction test and urease test^[11]. Susceptibility testing of *Acinetobacter* isolates for various antimicrobials was performed by Kirby Bauer disk diffusion method^[12]. The test organism was picked up with a sterile loop, suspended in peptone water and kept for incubation at 37°C for 2 hours. The turbidity of the suspension was adjusted to 0.5 McFarland's standard. Then the adjusted suspension was spread on the surface of a Mueller's-Hinton agar plate with a sterile cotton swab. The following antibiotic discs were then placed on the Mueller Hinton agar plate: Cotrimoxazole (25µg), Cefepime (30µg), Ciprofloxacin (5µg), Gentamicin (10µg), Amikacin (30µg), Doxycycline (30µg), Imipenem (10µg), and Meropenem (10µg), Ampicillin-Sulbactam (10/10µg). All dehydrated media and antibiotic disks were procured from HiMedia Labs Ltd(Mumbai India).The sensitivity and resistance of isolates was reported as per Clinical and Laboratory Standard Institute guidelines^[13]. Multi drug resistant (MDR) isolates were defined as those which depicted resistance to > 3 classes of antimicrobial tested^[14].

Determination of multiple antibiotic resistance (MAR) index

Multiple antibiotic resistance (MAR) index was determined for each isolate by using the formula $MAR = a/b$, where a represents the number of antibiotics to which the test isolate depicted resistance and b represents the total number of antibiotics to which the test isolate has been evaluated for susceptibility^[15].

Results

A total of 93 isolates of *Acinetobacter species* were recovered from various samples of patients admitted in clinical departments, out of which predominant were from urine 47(50.54%) followed by blood 27(29.03%), pus 7(7.53%) and endotracheal tube tip (ETT) 4(4.30%). Overall multi drug resistant (MDR) isolates were 57(61.29%) with majority from urine 35 (61.40%) and blood samples 8(14.04%). The overall antimicrobial susceptibility revealed Imipenem (75.27%), Meropenem (68.82%) and Doxycycline (68.82%) to be the efficacious drugs against *Acinetobacter* infections whereas high resistance was observed for other antimicrobials tested. Surprisingly, among the MDR isolates, 98.25% were resistant to Cotrimoxazole, 96.49% to Gentamicin and ciprofloxacin, 92.98% to Cefepime, 80.70% to Ampicillin/ sulbactam and 73.68% to Amikacin. The most promising drug in MDR *Acinetobacter* was Doxycycline (sensitivity 66.67%) followed by carbapenems namely Imipenem (sensitivity 61.40%) and Meropenem (52.63%) [Table 1]. MAR (Multiple antibiotic resistance) index revealed 71 isolates (76.34%) with MAR index greater than 0.2 and

22 (23.66%) isolates with MAR index less than 0.2. However, three isolates had shown MAR index of 01 (i.e. resistant to all the antimicrobials tested), out of which two were recovered from intensive care unit, and one from general surgery ward. [Table 2]. The *Acinetobacter* isolates which were having MAR index greater than 0.2 were mainly isolated from patients admitted in General surgery ward 20 (28.17%), intensive care units 19 (26.76%) and gynecology/

obstetrics ward 17 (23.94%) [Table 3]. Twenty six multi drug resistance patterns were observed for *Acinetobacter species* for the nine antimicrobials tested. Resistance to COT, CIP, GEN, AK, A/S, CPM, IMP, MRP (R8) was most frequently observed pattern in 8(14.04%) of MDR isolates. The other common pattern was resistance to COT,CIP, GEN, AK, A/S, CPM, DO (R7) observed in 5(8.77%) MDR isolates of *Acinetobacter spp* [Table 4].

Table 1: Antimicrobial susceptibility pattern of *Acinetobacter* isolates

Antibiotic	Total number of isolates n=93		MDR isolates n=57	
	Resistant	Sensitive	Resistant	Sensitive
Cotrimoxazole	70(75.27%)	23(24.73%)	56(98.25%)	01(1.75%)
Ampicillin/sulbactam	56(60.22%)	37(39.78%)	46(80.70%)	11(19.30%)
Doxycycline	29(31.18%)	64(68.82%)	19(33.33%)	38(66.67%)
Ciprofloxacin	64(68.82%)	29(31.18%)	55(96.49%)	02(3.51%)
Gentamicin	63(67.74%)	30(32.26%)	55(96.49%)	02(3.51%)
Amikacin	44(47.31%)	49(52.69%)	42(73.68%)	15(26.32%)
Cefepime	71(76.34%)	22(23.66%)	53(92.98%)	04(7.02%)
Meropenem	29(31.18%)	64(68.82%)	27(47.37%)	30(52.63%)
Imipenem	23(24.73%)	70(75.27%)	22(38.60%)	35(61.40%)

Table 2: MAR indices of *Acinetobacter* species (n=93)

MAR Index	Number
00	3(3.23%)
0.1	7(7.53%)
0.2	12(12.90%)
0.3	10(10.75%)
0.4	7(7.53%)
0.5	9(9.68%)
0.6	17(18.28%)
0.7	12(12.90%)
0.8	13(13.98%)
0.9	00
1.0	3(3.23%)

Table 3: Distribution of *Acinetobacter* isolates depending upon MARI value >0.2 among inpatients

Department	Number of isolates with MAR index >0.2 (n=71)	Percentage (%age)
Intensive care unit	18	26.76%
General Surgery ward	20	28.17%
Orthopedics	01	1.41%
Gynecology / obstetrics	18	23.94%
General Medicine ward	08	11.27%
Respiratory Medicine	03	4.23%
Pediatrics	03	4.23%
Total	71	100%

Table 4: Resistance pattern observed in MDR *Acinetobacter* isolates (n=)57

Resistance pattern	Number
COT, CIP, GEN, CPM (R4)*	2 (3.51%)
COT, CIP, GEN, MRP (R4)*	1(1.75%)
COT, CIP, GEN, DO (R4)*	1(1.75%)
COT, CIP, GEN, A/S, CPM (R5)#	4(7.02%)
COT, CIP, GEN, CPM, DO (R5)#	1(1.75%)
COT, CIP, GEN, AK, CPM (R5)#	1(1.75%)
COT, CIP, GEN,AK ,A/S (R5)#	1(1.75%)
COT, GEN, CPM, A/S, DO (R5)#	1(1.75%)
COT, CIP, GEN, AK,A/S,CPM (R6)**	4(7.02%)
COT, CIP,GEN,AK,CPM,MRP (R6)**	3(5.26%)
COT, GEN,AK,CPM,A/S,IMP (R6)**	2(3.51%)
COT, CIP,GEN, AK, CPM, MRP (R6)**	2(3.51%)
COT, CIP, GEN, AK,A/S,IMP (R6)**	1(1.75%)
COT,CIP,GEN,CPM,MRP,DO (R6)**	1(1.75%)
COT, CIP, GEN,AK, MRP,DO (R6)**	1(1.75%)
CIP,GEN,AK,CPM,A/S,DO (R6)**	1(1.75%)
COT,CIP,CPM,A/S,MRP,DO (R6)**	1(1.75%)
COT,CIP, GEN, AK, A/S, CPM, DO (R7)##	5(8.77%)
COT,CIP, GEN, AK, A/S, CPM, IMP (R7)##	4(7.02%)
COT,CIP,GEN, AK, CPM, A/S,MRP (R7)##	3(5.26%)
COT, CIP, GEN, CPM, A/S, IMP, MRP (R7)##	1(1.75%)
COT, CIP, GEN, AK, CPM, A/S, IMP, DO (R7)##	2(3.51%)
COT, CIP, GEN, AK, A/S, CPM, IMP,MRP (R8)°°	8(14.04%)
COT, CIP, GEN, AK, CPM, A/S, MRP, DO (R8)°°	2(3.51%)
COT, CIP, GEN, CPM, A/S, IMP, MRP, DO (R8)°°	1(1.75%)
COT, CIP, GEN, AK, A/S, CPM, IMP, MRP, DO (R9)#9	3(5.26%)
TOTAL	57

* R4; # R5; ** R6; ## R7; °° R8; #9 R9

COT (Cotrimoxazole); CIP(Ciprofloxacin); GEN (Gentamicin); AK (Amikacin); A/S (Ampicillin/sulbactam); CPM(Cefepime); IMP (Imipenem); MRP (Meropenem); DO (Doxycycline)

Discussion

Despite intensive efforts, nosocomial acquisition of multi-drug resistant (MDR) *Acinetobacter baumannii* is still a problem due to its great ability to disseminate from and colonize human and environmental reservoirs^[16]. In present study *Acinetobacter species* was recovered from 93 inpatients. The isolates were predominantly from urine (50.54%) which is similar to findings Lahiri KK et al, who documented 51.97% urinary isolates of *Acinetobacter spp*^[17], followed by blood (29.03%) samples. The study estimated MDR isolates of *Acinetobacter spp.* as 57(61.29%), which is in agreement with study of Sivaranjani V et al., and Dash et al., who found multi drug resistance in 71.31% and 54.7% of *Acinetobacter spp* isolates respectively^[5,6]. Majority of MDR isolates (61.40%) were found in urine specimens which is similar to Lahiri KK et al. who found 63.2% multi drug resistant isolates in urine specimens^[17]. Multiple antibiotic resistance (MAR) in bacteria is most commonly associated with the presence of plasmids which contain one or more resistance genes,

each encoding a single antibiotic resistance phenotype. High prevalence of multidrug resistance indicates a serious need for broad-based, local antimicrobial resistance surveillance and planning of effective interventions to reduce multidrug resistance in such pathogens^[9]. The overall antibiotic susceptibility pattern depicted that *Acinetobacter spp.* being highly resistant to commonly used antibiotics. The resistance rate of *Acinetobacter* to various antibiotics was Cotrimoxazole (75.27%), Ciprofloxacin (68.82%), Gentamicin (67.74%), Ampicillin/sulbactam (60.22%) and Cefepime (76.34%), which is consistent with findings of other workers^[5,18]. With the exception of Imipenem, Meropenem and Doxycycline, susceptibility of *Acinetobacter* to other antibiotics was low in our hospital setting. In our study, 75.27% isolates were susceptible and 24.73% resistant to Imipenem, which agrees with study done at Indore, in which 65.5% of the isolates were susceptible to Imipenem^[19]. These findings are also concordant with a study from Mumbai that showed 29% of clinical isolates of *Acinetobacter spp.* resistant to Imipenem^[20]. A study from Spain reported that 43% of *A. baumannii* isolates were resistant to Imipenem^[21]. One report from the U.S.A has documented Imipenem resistance of 23.1% in *Acinetobacter baumannii* in their study^[22]. The regional

variation in resistance of *A. baumannii* to Imipenem is related to pattern of antimicrobial use and associated risk factors^[19]. Other antimicrobials which exhibited in vitro activity against *Acinetobacter spp.* were Meropenem and Doxycycline with similar sensitivity rate of 68.82%. As shown in this study MDR *Acinetobacter* was highly resistant to most of the antimicrobials tested. Doxycycline remained promising drug in MDR *Acinetobacter* with susceptibility of 66.67% as compared to carbapenems, Imipenem (61.40%) and Meropenem (52.63%). Literature data confirmed the findings of this study especially the efficacy of association of Doxycycline as a therapeutic strategy^[23,24]. Khan F et al have quoted that except Imipenem, Doxycycline was found to have the best spectrum of activity against *Acinetobacter* with just 48.34% (73 isolates) resistance which shows that Doxycycline have retained the in vitro activity against multidrug resistant isolates^[25]. Vila et al. studied the susceptibility of 54 *A. baumannii* to antimicrobial drugs in which 98% of the strains were susceptible to Doxycycline respectively^[26]. The low level of resistance observed for Doxycycline would probably be associated to the limited prescription of these drugs in study region. The doxycycline is a broad-spectrum antibiotic oxytetracycline synthetic derivative used in several countries. Compared to other oral tetracyclines, it has the best pharmacokinetic and safety profile. Doxycycline is a relatively well-tolerated drug in the tetracycline class. Although the most common adverse events described for Doxycycline include the esophageal erosion and photosensitivity, it is contraindicated in several groups, including those with allergy/sensitivity to the drug, pregnant and lactating women and young children. It has better absorption, longer half-life, better penetration and bioavailability. Since Doxycycline is well absorbed and have half-lives of 16 to 18 hours, less frequent and lower doses are possible^[25]. The proportion of isolates with MAR index greater than 0.2 was 76.34% (71) while those had multiple antibiotic resistance (MAR) index of 0.2 or less was 23.66% (22). These findings reflect that a greater proportion of the isolates are likely to be from high risk source and originate from an environment where several antibiotics are used^[27]. In current study most of the MDR isolates and those with MAR index greater than 0.2 were found from general surgery ward (28.17%) followed by intensive care units (26.76%) and gynecology/obstetrics ward (23.94%) whereas Sivaranjani V et al., observed MDR *Acinetobacter* isolates mainly from intensive care units (42.53%) followed by general surgery (26.44%) and gynecology/obstetrics units (17.24%)^[5]. Compared to other methods of bacteria source tracking such as genotypic characterization, the MAR indexing method is cost effective, rapid and easy to perform. It is also simple and does not require specialized training and expensive equipment. The monitoring of both antibiotic

consumption and multiple antibiotic resistances (MAR) especially in nosocomial infections is critically necessary to effective containment programs and audit of such programs^[9].

Conclusion

Antimicrobial resistance is growing menace worldwide. Infections with multi drug resistant *Acinetobacter* has resulted in limited choice of antimicrobials for treatment of hospitalized patients. Doxycycline has exhibited efficacy against MDR *Acinetobacter*, in current study, which can be considered as alternative therapy apart from carbapenems. *Acinetobacter species* are ubiquitous organisms which survive for long periods in the hospital environment. Moreover the pathogen has considerable selective advantage in environments with widespread and massive use of antibiotics. As antimicrobial resistance is a growing global problem so periodic monitoring of resistance pattern of *Acinetobacter* species among inpatients is beneficial to the patient as well as clinician in selection of chemotherapy.

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