Original Article

Doxycycline sensitivity in multidrug resistant *Acinetobacter baumanii* isolates from blood

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Keywords: *Acinetobacter, Blood, Doxycycline, Resistance*

Abstract

Background: *Acinetobacter* species has become a leading cause of blood stream infection in health care setting. *Acinetobacter* species possess a wide array of β-lactamases that hydrolyze and confer resistance to penicillins, cephalosporins and carbepenems. Doxycycline, a semisynthetic tetracycline, has effective antibacterial effect on multidrug resistant *Acinetobacter* species.

Methods: Total numbers of 13,880 samples were received in 5 years for blood culture in brain heart infusion broth. Antimicrobial susceptibility testing was done on Mueller Hinton's agar by Kirby Bauer Disc diffusion method as per the Clinical and Laboratory Standards Institute (CLSI) guidelines for the following antimicrobials: cefotaxime 30µg, ceftriaxone 30µg, cefoperazone 75µg, cefipime 30µg, cefoperazone+sulbactum 75/75 µg, gentamicin 10µg, amikacin 30µg, netilmicin 30µg, tobramycin 10µg ,ciprofloxacin 5µg, piperacillin+tazobactum 100/10 µg , Imipenem 10µg and doxycycline 30µg. Isolates resistant to atleast three drugs belonging to three different groups were considered to be multidrug resistant (MDR).

Results: One hundred and fifty one isolates were identified as *Acinetobacter baumanii*. Majority of the isolates 101(66.88%) were from the patients between 0-10 years of age. 45% of the isolates were found to be multidrug resistant. Barring imipenem, doxycycline was found to have the best spectrum of activity with just 48.34% (73 isolates) resistance

Conclusion: Rational and appropriate use of antimicrobial agents is of paramount importance to minimize the risk of resistant organism. Doxycycline can be used as an effective therapeutic agent in multidrug resistant *Acinetobacter baumanii*.

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1. Introduction

Acinetobacter baumanii, a non-fermentative, aerobic, opportunistic, gram-negative cocco bacilli has emerged as a very important nosocomial pathogen and mainly affects patients with impaired host defences in intensive care units and is responsible for many hospital outbreaks.[1] Its ability to chronically colonize patients and cause outbreaks which are usually hard to eradicate poses significant challenge to infection control and increases healthcare expenditure.[2] It is implicated in a variety of nosocomial infections like blood stream infections, pneumonia, meningitis, urinary tract infections, skin and soft tissue infections, wound and burn infections, intravascular devices and implant related infections.[3] *Acinetobacter* species has become a leading cause of blood stream infection in health care setting. Nosocomial *Acinetobacter baumannii* bloodstream infections occur with significant prevalence and mortality.[4] Acinetobacter species possess a wide array of β-lactamases that hydrolyze and confer resistance to penicillins, cephalosporins and carbepenems. Besides resistance to the β-lactam antimicrobials, resistance to other classes of antibiotics is almost always present in the *Acinetobacter* species.[5] The increasing development of drug resistance severely restricts the therapeutic options available for the treatment of patients with such infections. The emergence of drug resistance in *Acinetobacter* species also leads to increased length of stay in hospitals and higher mortality rates.[6] Doxycycline is an antibiotic not commonly used for *A. baumannii* infections, but to which a high percentage of *A. baumannii* strains are susceptible.[7] Owing to the frequency of imipenem-resistant strains and the need to find new therapeutic approaches, we have compared antibiotics used frequently in infections caused by this organism, with doxycycline. Doxycycline, a semisynthetic tetracycline has effective antibacterial effect on multidrug resistant *Acinetobacter* species. The present study was done to study the prevalence of *Acinetobacter* species and their antimicrobial sensitivity pattern in patients with blood stream infections. The *in-vitro* activity of doxycycline for treatment of *Acinetobacter* species was also evaluated.

2. Materials and Methods

The study was done in the Enteric laboratory of the Department of Microbiology, Jawaharlal Nehru Medical College and Hospital, Aligarh from January 2005-December 2010.Total number of 13,880 samples were received in 5 years for blood culture in brain heart infusion broth. The study was approved by the institutional ethical committee and informed consent was taken from all patients before collection of blood sample. Repeated subcultures were done on 5% sheep Blood agar and Mac-Conkeys agar after 24 hours, 48 hours and 7 days of incubation at 37˚C.Cultures showing growth were identified by standard biochemical procedures.[8] Antimicrobial susceptibility testing was done on Mueller Hinton's agar by Kirby Bauer Disc diffusion method as per the Clinical and Laboratory Standards Institute (CLSI) guidelines,[9] for the following antimicrobials: cefotaxime 30µg, ceftriaxone 30µg, cefoperazone 75µg , cefipime 30 µg, cefoperazone-sulbactum 75/75 µg, gentamicin 10µg, amikacin 30µg, netilmicin 30µg, tobramycin 10µg, ciprofloxacin 5µg, piperacillin+ tazobactum 100/10 µg, Imipenem 10µg and doxycycline 30µg. Manchanda et al. defined isolates resistant to atleast three drugs belonging to three different groups to be multidrug resistant (MDR).[10] Screening of possible ESBL production was done by using ceftriaxone (30µg) and cefoperazone (75µg). Those isolates with zone diameters less than 25mm for ceftriaxone and less than 22mm for cefoperazone were subsequently confirmed for ESBL production. Confirmation was done by noting the potentiation of the activity of cefoperazone in the presence of cefoperazone sulbactum.[9] Detection of AmpC betalactamase was done for isolates resistant to ceftriaxone (30µg), cefoperazone (75µg) and cefoperazone -sulbactum (75/75µg). Induction of AmpC synthesis was based on the disc approximation assay using imipenem as inducer. [11]

3. Result

A total of 2160 samples were positive on culture over a period of five years. One hundred and fifty one isolates were identified as *Acinetobacter baumanii*. Majority of the isolates 101(66.88%) were from the patients between 0 - 10 years of age (Table 1 and Figure 1). On antimicrobial sensitivity testing maximum resistance was shown to the β-lactam group of antimicrobials (90.81%). Flouroquinolones and aminoglycosides also had a poor activity with resistance to 71.43% and 79.15% of the isolates. All the isolates were uniformly sensitive to imipenem. Isolates found to be multidrug resistant were 83.45% in number. Cefipime showed 100% resistance for all five years. Barring imipenem, doxycycline was found to have the best spectrum of activity with just 48.34% (73 isolates) resistance (Figure 2). ESBL producing isolates were 10(13.9%) and 11(15.9%) isolates were AmpC producers.

Table1: Pattern of *Acinetobacter* species isolated in bloodstream infections in relation to different wards

WARDS	NO. OF ISOLATES					
	2005	**2006**	**2007**	**2008**	**2009**	**2010**
Medicine	4(10.5%)	3(10.7%)	6(30%)	3(12.5%)	1(5,8%)	3(12.5%)
Pediatrics	27(71%)	19(50%)	10(50%)	20(83.3%)	12(70.5%)	20(83.3%)
Surgery	3(7.8%)	5(13.1%)	3(15%)	1(4.1%)	4(23.5%)	1(4.1%)
Gynecology	4(10.5%)	1(2.6%)	1(5%)	0(0%)	0(0%)	0(0%)
Orthopedics	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Total	38	28	20	24	17	24

Most of the patients were in pediatric age group

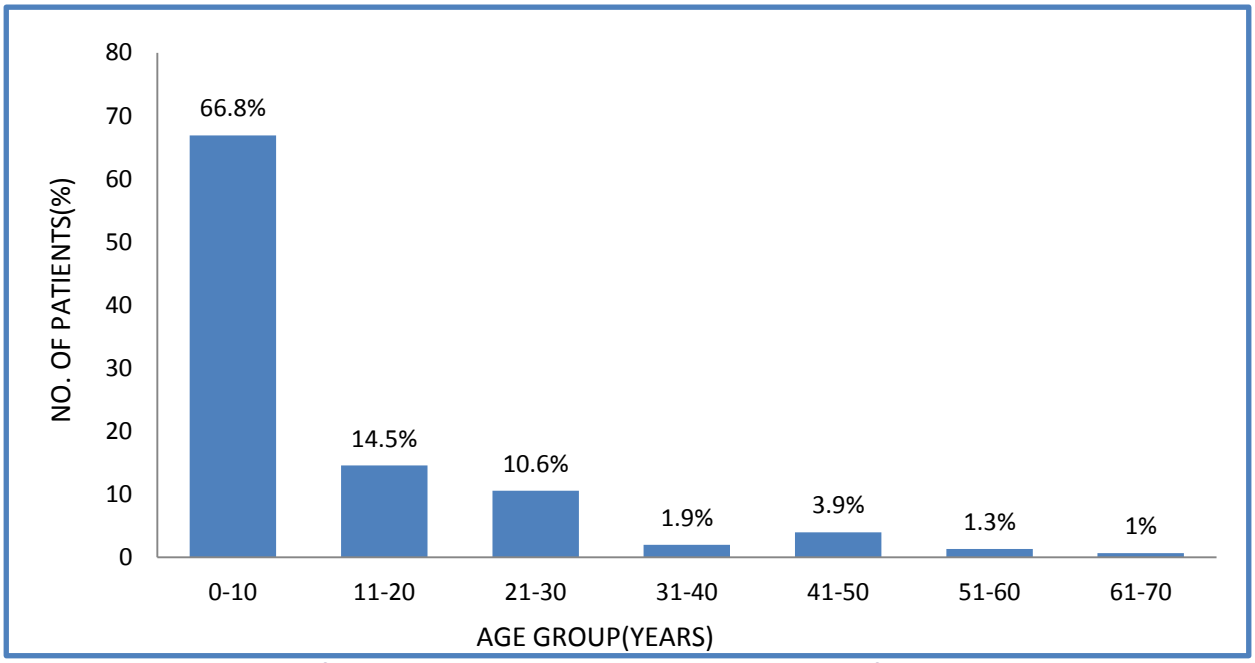

Figure 1: Pattern of Acinetobacter species isolated in bloodstream infections in relation to age.

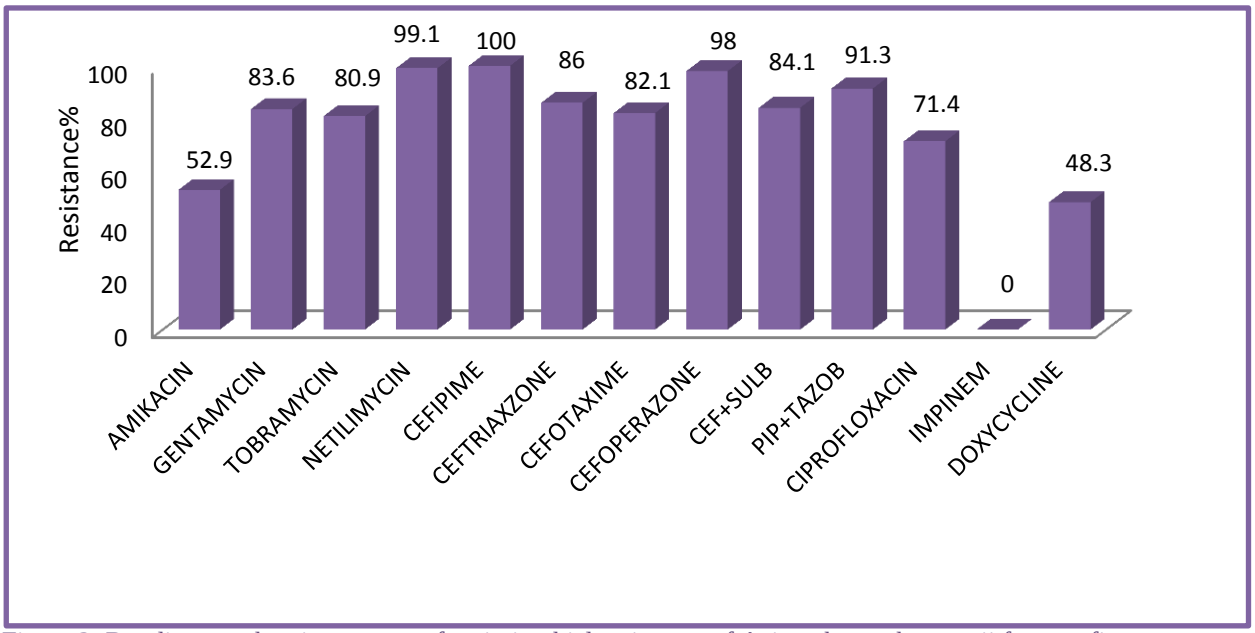

Figure 2: Bar diagram showing pattern of antimicrobial resistance of Acinetobacter baumanii for past five years.

4. Discussion

Acinetobacter species are rapidly spreading pathogens with emergence of extended resistance to almost all the antimicrobial agents. The fluoroquinolones, aminoglycosides and especially the β-lactam antimicrobials can no longer be recommended for the treatment of patients with *Acinetobacter* bacteremia because of the high level of resistance. Imipinem and tetracycline are among the most active drugs against multidrug resistant *Acinetobacter baumanii*. Although imipenem at present have a good spectrum but resistance to imipenem is also coming up in different regions of India and other parts of the world.[10] In a surveillance done in Taiwan, it was found that the prevalence of imipinem resistant *Acinetobacter baumanii* increased from 3.4% in 2002 to 58.7% in 2010.[12] Infections produced by multiresistant *A. baumannii* strains have an attributable mortality of 25–34%;the use of inappropriate treatment being a factor associated with poor prognosis. Imipenem and tetracycline are among the most active drugs against multiresistant *A. baumannii*.[7]

In our study, maximum resistance was shown to the β-lactam group of antimicrobials (90.81%). Flouroquinolones and aminoglycosides also had a poor activity with resistance to 71.43% and 79.15% of the isolates. All the isolates were uniformly sensitive to imipenem. 83.45% of the isolates were found to be multidrug resistant. Except imipinem, doxycycline was found to have the best spectrum of activity with just 48.34% (73 isolates) resistance which shows that doxycycline have retained the in vitro activity against multidrug resistant isolates. Other studies done on Acinetobacter species also revealed that doxycycline had better sensitivity pattern against multidrug resistant isolates.

Vila et al. studied the susceptibility of 54 *A. baumanii* to antimicrobial drugs in which 98% of the strains were susceptible to doxycycline respectively. Only 55% of the strains were sensitive to ceftazidime and 52% to ampicillin/sulbactum.[7] In a study in Cairo, Egypt, the antimicrobial susceptibility patterns showed that 20 *A. baumannii* isolates tested were totally resistant to imipenem, ampicillin/sulbactam, ceftazidime, ciprofloxacin, piperacillin/tazobactam and ceftriaxone. A high resistance rate was observed to amikacin trimethoprim/sulfamethoxazole (90% each) and gentamicin (85%) while least resistance was noted for doxycycline (75%).[13] In another study in Pune, 90% of the isolates of *A. baumannii* were resistant to minimum of 23 antibiotics showing 95-100% resistance to β lactum group of antibiotics and only 85% resistance to doxycycline.[14] Another study which evaluated the antimicrobial susceptibility to tetracycline, minocycline, doxycycline and by E-test. Of all isolates, 89% were resistant to tetracycline. Minocycline with the resistant rate of 35% and Doxycycline with the resistant rate of 25% have a good activity against *A. baumannii* isolates.[15]

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 oesophageal erosion and photosensitivity, it is contraindicated in several groups, including those with allergy/sensitivity to the drug, pregnant and lactating women and young children. [16] 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.[17]

5. Conclusion

Rational and appropriate use of antimicrobial agents is of paramount importance to minimize the risk of resistant organism. Doxycycline can be used as an effective therapeutic agent in multidrug resistant *Acinetobacter* baumanii. There is a need to study global epidemiology of multidrug-resistant *A. baumannii* using molecular typing of bacterial isolates and characterization of antibiotic resistance in order to control the spread of *A. baumannii* infections over a wide geographic region.

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Competing Interests

None declared.

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