Prevalence of tigecycline resistant multidrug resistant *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex from a tertiary care hospital in Nepal

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ABSTRACT

Acinetobacter calcoaceticus-Acinetobacter baumannii (ACB) complex is an important nosocomial pathogen. Rapid development of resistance to multiple drugs limits the therapeutic options for the treatment of infections by ACB complex. The current study aimed to find the prevalence of ACB complex and know the antibiogram of these clinical isolates including the rate of multidrug resistance (MDR) and the in vitro activity of tigecycline against these isolates. This study was conducted from January to August, 2015. Of the 1,196 bacteria isolated from 10,950 clinical specimen, 150 (12.5%) were ACB complex. MDR-ACB complex were 71.3% of which 73.3% were from inpatient source. Resistance rates for ceftazidime 81.3%, ampicillin/sulbactam 56.7%, amikacin 56.7%, ciprofloxacin 68.7%, imipenem 47.3%, piperacillin 80.7% and piperacillin/tazobactam 68% were seen. Almost all ACB complex were sensitive to polymixin B and 98% to colistin. However, adopting the breakpoints recommended by the US FDA for Enterobacteriaceae, the resistance rate for tigecycline was 37.3%. Thus the present study provides valuable information regarding the very high prevalence of ACB complex in our hospital; the alarming number of MDR- ACB complex and the worrisome finding of comparatively high resistance rate to the latest drug- tigecycline.

Keywords: *Acinetobacter calcoaceticus-Acinetobacter baumannii* (ACB) complex, multidrug resistance, tigecycline, Nepal

INTRODUCTION

Acinetobacter calcoaceticus – Acinetobacter baumanii (ACB) complex is emerging as the most common and worrisome nosocomial pathogen. ACB complex comprises of a group of bacteria which are non fermenting, aerobic, Gram negative coccobacilli.[1-3] Their ubiquitous presence, survival ability and rapid development of resistance to commonly used antimicrobials have made them a successful nosocomial pathogen.[4]

Various studies from different parts of the world have reported an increasing number of multidrug resistant (MDR) strains of ACB complex.[5] Some have even encountered strains resistant to the 'last-resort' drugs like rifampicin, tigecycline, polymixin B and colistin sulfate.[6]

In this study we have analyzed the prevalence of ACB complex in our setting; the percentage of MDR-ACB isolates and their sensitivity towards tigecycline, polymixin B and colistin sulfate.

MATERIALS AND METHODS

A hospital based prospective cross sectional study was performed in the microbiology laboratory of Nepal Medical College Teaching Hospital (NMCTH), Kathmandu, Nepal. The study was conducted from January to August, 2015. Ethical approval was received from the institutional review board. All clinical samples, appropriately collected, labeled and transported with no visible contamination and processed for aerobic bacterial culture were included in the study. Samples from all age groups and both the sexes were included. These were processed according to standard microbiological procedures.[7] Briefly, samples were examined macroscopically, followed by gram staining (except for blood and urine) and were inoculated on appropriate culture media. They were then incubated aerobically at 35^{0}C for 18-24 hours. Blood cultures were retained for 3 days. Bacterial growths were identified by studying the colony morphology, Gram staining and biochemical reactions.

Antibiotics susceptibility test for ceftazidime (30µg), cefotaxime (30µg), ceftriaxone (30µg), piperacillin (30µg), pipercillin/tazobactam (100/10µg), ampicillin/sulbactam (10/10µg), ciprofloxacin (5µg), amikacin (30µg), imipenem (10µg), tigecycline (15µg), colistin (20µg) and polymyxin B (300units), were performed according to Clinical Laboratory Standard Institute (CLSI) guidelines.[8] The breakpoints proposed by the U.S. Food and Drug Administration (FDA) were used to interpret the susceptibility for tigecycline (Tygacil package insert [June

2005], Wyeth Pharmaceuticals Inc., Philadelphia, PA).

The data obtained were analyzed using SPSS version 21.

RESULTS

A total of 10,950 clinical samples received for aerobic bacterial culture and antibiotics susceptibility testing at NMCTH from January to August, 2015 were included in the study. Out of the 1,196 bacterial isolates, 984 were gram negative bacilli and 212 were Gram positive cocci. ACB complex was isolated from 150 samples. It was the third most commonly isolated Gram negative bacteria from clinical samples. The prevalence of ACB complex among total isolates was 12.5% and among gram negative bacilli was 15.3%. Ninety percent of these were from the inpatient source. The rate of isolation of ACB complex from male and female patients was almost equal (50.6% vs 49.4%). The highest numbers of ACB complex were isolated from samples from patients between 21 -30 years of age (22.7%). The most common sample to yield ACB complex was sputum (28%) followed by blood (24%), urine (11.3%), endotracheal tube (11.3%), pus (10.7%), wound swab (7.3%), body fluid (2.7%) and others (4.7%). The antimicrobial susceptibility pattern of the ACB complex is shown in Table 1.

Table 1: Antimicrobial susceptibility pattern of ACB complex

Antibiotics used	Resistant (%)	Sensitive (%)
Amikacin	85 (56.7%)	65 (43.3%)
Ampicillin/sulbactam	85 (56.7%)	65 (43.3%)
Cefotaxime	101 (67.3%)	49 (32.7%)
Ceftazidime	122 (81.3%)	28 (18.7%)
Ceftriaxone	104 (69.3%)	46 (30.7%)
Ciprofloxacin	103 (68.7%)	47 (31.3%)
Colistin	3 (2%)	147 (98%)
Imipenem	71 (47.3%)	79 (52.7%)
Piperacillin	121 (80.7%)	29 (90.3%)
Piperacillin/tazobactam	102 (68%)	48 (32%)
Polymixin B	1 (0.7%)	149 (99.3%)
Tigecycline	56 (37.3%)	94 (62.7%)

DISCUSSION

The prevalence of ACB complex among clinical isolates in our study was 12.5%. Similar prevalence was reported from other hospitals from Nepal.[9] Although the figure may not seem to be alarming, ACB complex is notorious for its rapid spread and acquisition of drug resistance. In this study, it was the third most common Gram negative bacilli following *Escherichia coli* and *Klebsiella pneumoniae*. The fact that 90% of ACB complex were from inpatient source highlights that this nosocomial pathogen has carved its niche in our hospital as well. Inpatients make a high risk group for ACB complex infection since they are either immunocompromised by their disease per se or due to the treatment they are receiving or they have undergone some invasive procedure or have indwelling devices.[10] All these and the hospital environment (patients and care givers colonized with ACB complex and the contaminated surfaces and articles that come in patient contact) favor ACB complex infection to the at risk patients.[11] Results from studies from other hospitals support our findings.[12-13]

The ACB complex can infect any system of our body. In the present study, the highest number of isolation of ACB complex was from respiratory tract sample (sputum) followed by blood (28% and 24% respectively). The differences in the sample types, size, source, site of study, antibiotics usage, and the hospital infection control practices followed can influence the rate of isolation from different clinical specimens.[9, 12, 14]

The same factors may also play a role in difference in the antibiogram of the isolates from different hospitals. In the present study, a high resistance rate (81.3%) to ceftazidime was noted. Similar high resistance rates to this drug were reported from other hospitals from Nepal [82.3% from Tribhuvan University Teaching Hospital (TUTH) [13] and 84% from B.P Koirala institute of health sciences (BPKIHS) [12]. Although resistance to betalactam-betalactamase inhibitor combination drug was lower than that to betalactam alone, the resistance rate was high enough to raise concern: 56.7% to ampicillin/sulbactam and 68% to piperacillin/tazobactam. Similar findings were reported from BPKIHS[12] with 69% of their ACB complex resistant to piperacillin/tazobactam.

Aminoglycoside and quinolone resistance are also common place.[13] The bacterium utilizes plasmid or transposon coded aiminoglycoside modifying enzymes,[15] efflux pumps, alteration in target ribosomal protein and ineffective transport to interior of bacteria.[16] Amikacin resistance was detected in 56.7% of the ACB complex from our study. Chromosomal mutations altering the target enzymes DNA gyrase and topoisomerise IV; efflux pump and acquisition of mobile genetic element are mechanisms by which ACB complex acquired resistance to quinolones.[17] We encountered 68.7% ACB complex resistant to ciprofloxacin. Another study from tertiary care hospital in Kathmandu, Nepal has reported 64.5% ACB complex as resistant to ciprofloxacin.[13]

After the widespread resistance to commonly used drugs, carbapenems were the drug of choice for managing

Acinetobacter infection in the late 1990s. They have low toxicity and good activity against ACB complex.[18] Unfortunately, however, carbapenem resistant strains are increasingly being reported worldwide.[19, 20] We report 47.3% of ACB complex showing resistance to imipenem. In a study conducted in Greece between 1996 and 2007, carbpenem resistance increased from 0 to 85.1% [21] and in the United Kingdom (UK), the resistance rate increased from 0 to 55% between 1998 and 2006.[22] The resistance rate was higher where carbapenems were extensively used like in Greece, Turkey and Italy and lower in countries where their use was less for example in Holland and Scandinavian countries.[23] The resistance to β lactam antibiotics including carbapenems is acquired mainly by the production of betalactamases (chromosomally mediated; plasmid or transposon borne)[24] and also by non-enzymatic mechanism like changes in the outer membrane proteins, multidrug efflux pumps, alterations in penicillin binding proteins[17]. Carbapenems are widely and indiscriminately prescribed in Nepal which explains the high resistance rate to these drugs.

ACB complex is notorious for its multidrug resistance (MDR). The rapid emergence and spread of MDR strains may be due to the combined effect of upregulation of its innate resistance mechanism coupled with gene acquisition following lateral gene transfer and clonal spread of MDR clones.[25, 26] This process is aided by selective pressure exerted by the use of broad spectrum antibiotics and transmission of strains among patients. In the present study, 71.3% ACB complex were MDR. Variable rates of MDR- ACB complex are reported by other researchers from Nepal.[13, 27] Besides the differences in the study setting and study population, non uniformity in the definition of MDR could be the reason for the variation in the percentages of MDR isolates. In the present study, MDR- ACB complex were those which showed resistances to three or more of the following drugs: antipseudomonal cephalosporins (ceftazidime or cefepime), antipseudomonal carbapenems (imipenem or meropenem), ampicillin-sulbactam, fluoroquinolones (ciprofloxacin) and aminoglycosides (amikacin).[28] All of these studies however confirm to the fact that inpatients harbor more MDR than outpatients.[12, 13] Our study had 73.3% MDR from inpatient source. Inappropriate use of cabapenems and other broad spectrum antibiotics is responsible for the global rise in prevalence of MDR-ACB complex.[29] A study from Iran during 2001-2011 showed MDR *A. baumannii* increase from 50% to 94%.[30]

MDR in ACB- complex limits the therapeutic choices in the treatment of infection caused by this bacterium. Evolution of strain resistant to carbapenem as well has further narrowed the treatment options. Rifampicin, tigecycline, colistin and polymyxin are considered the last resort drugs. However, increasing resistance rates and higher minimum inhibitory concentration are being reported for tigecycline.[31] Emergence of resistance during treatment has been reported [32] probably mediated by efflux pumps.[31] Although tigecycline came in widespread use in our country only later, we report a higher percentage (37.3%) of ACB complex resistant to it than those reported from places where it was introduced earlier, for example, 2.7% from the UK[33] and 26% from Argentina[34]. Tigecyline, a novel broad spectrum glycylcycline was approved by FDA and the European Medicines Agency for the treatment of complicated skin and intraabdominal infections.[35] However, the definitive susceptibility breakpoints for *Acinetobacter* genus have not been published by either CLSI or FDA. Jones et al[36] reported using the breakpoint zone diameter of ≥16/≤12 mm to define susceptibility/ resistance respectively, instead of those proposed by U.S. FDA for Enterobacteriaceae family organisms (≥19/≤14 mm) reduced the intermethod minor errors to an acceptable level (only 9.7% instead of 23.3% with FDA breakpoints proposed). Studies showed a significant reduction in the resistance rate when Jone's proposed breakpoints are adopted instead of the FDA breakpoints {(3% vs 26%) [34]; (3.57% vs 22.61%) [37]}. Similar reduction in the resistance rate is expected in our study if the interpretive criteria proposed by Jones are followed. This has significant clinical implication in the treatment of MDR-ACB complex. Since almost all the diagnostic laboratories in Nepal rely on the disc diffusion method as the sole method for antimicrobial susceptibility testing, definitive tigecycline breakpoints for ACB complex is still awaited.

Colistin was used clinically because of its proven ability to treat infections caused by MDR- *A baumannii* and other MDR organism.[38] Many studies have reported cure rates or improvement with colistin of 57-77% among severely ill patients with MDR *Acinetobacter species* infection.[24] The ACB complex isolated at various places still show a high susceptibility to colistin. In our study 98% were susceptible. Similarly, 97% and 98% of isolates from studies from Greece and UK were susceptible.[21] The European arm of the SENTRY surveillance program identified 2.7% of polymyxin B resistant *A. baumannii* isolates collected during 2001-2004;[39] one of our isolates was resistant to polymyxin B.

The present study establishes ACB complex as an important nosocomial pathogen in our hospital. With the emergence of extended resistance even to newer antimicrobials, we are headed towards the 'pre antibiotics era'. Irrational use of antibiotics, absence of antimicrobial stewardship programme in hospitals, lack of surveillance and reporting system, failure to observe infection control practices like hand washing and barrier nursing could be some reasons for this problem. Urgent interventions are required to check the spread and delay the emergence of resistance in MDR-ACB complex.

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