Research Article

BJMP 2015;8(1):a802

Trend of developing resistance among isolates of Acinetobacter spp.; Threat of hospital acquired infection

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Abstract

Aim: Acinetobacter sp. is a Gm-ve bacteria which is a major cause of serious infections. Today it has emerged as multidrug resistant organism. The aim of current study was to evaluate the trend of sensitivity/resistance pattern of Acinetobacter spp. against broad spectrum antibiotics.

Method: Standard Kirby bauer Disc Diffusion method was adopted to conduct the study according to the CLSI 2013 Standards. Total 52 isolates were collected from different sites of inpatients admitted to renowned tertiary care hospital from Feb 2014-March 2014 and sensitivity/resistance pattern was observed against 08 broad spectrum antibiotics of different classes.

Result: It is observed that 61.5% of all samples were obtained from male patients while, the mean range of age group among both the gender frequently found infected was 51-75 yrs. The highest percentage of isolate was obtained from tracheal aspirate (55.76%) of both the genders. Both Colistin and Polymixin were found to be most effective against 98% isolates each, while Imipenem was the least effective broad spectrum antibiotic. Thus, the isolates were highly resistant to 05 antibiotics traditionally used to treat infections caused by Acinetobacter spp. Surprisingly, more than 32% of isolates showed Intermediate sensitivity to Fosfomycin.

Conclusion: Due to emerging trend of developing resistance among Acinetobacter spp. and spread of hospital acquired infections. There is a serious need to take necessary steps by hospital officials to ensure cleanliness. Patients should also be educated about the proper use of antibiotics.

Keywords: Acinetobacter sp. , Hospital acquired, Resistance Pattern.

Abbreviations: CLSI :Clinical and Laboratory Standards Institute

Introduction

For decades the genus *Acinetobacter* has undergone several taxonomical modifications. Large number of non-fastidious, aerobic, Gram-negative bacteria (GNB) are included in this genus. In the last few years these organisms are genetically modifying into highly resistant forms resulting in untreatable nosocomial infections[1] and health care associated infections.[2] *Acinetobacter* is also a major cause of invasive type infections in children resulting in untreatable urinary tract infections (UTIs), skin infections and septicemia.[3] One identified cause of the resistance mechanism in carbapenem resistant *Acinetobacter* spp. is the production of the MBL enzyme.[4] It has been revealed through various published studies that *Acinetobacter* displays a specific type of mechanism of resistance against different antimicrobials. Some of them, for example β-lactam, are inhibited by enzymatic degradation, while quinolones are rendered ineffective due to a genetic mutation preventing the binding of an antibiotic to a distinct binding site. The same is true with aminoglycosides in which the resistant strains are noticed to acquire a gene involved in enzymatic modification.[1]

Although polymixin resistance in *Acinetobacter* spp. was reported the specific cause of resistance was unknown until 2008. In 2013, one study detected the presence of hetero-and adaptive resistance due to mutation in specific gene for the first time.[1,21] Hence the aim of this current study was to evaluate the trend of sensitivity/resistance pattern of*Acinetobacter* spp. against broad spectrum antibiotics.

Method and Materials

The objective of the study was to evaluate the sensitivity of *Acinetobacter* spp. to 08 broad spectrum antibiotics. The Kirby Bauer Disc Diffusion method was used following the standard procedures as laid down by CLSI 2013.[6] A total of 52 isolates were collected from Feb 2014-March 2014 from patients admitted to tertiary care hospitals in Karachi. The isolates were identified by routine lab procedures.

Antimicrobial agents and medium: Standard (Oxoid) discs of Amikacin (30 µg), Cefoperazone (75 µg), Ceftriaxone (30 µg), Ciprofloxacin (5 µg), Colistin (10µg), Fosfomycin (50 µg), imipenem (10 µg), Polymixin B (300units), Mueller Hinton Agar (Oxoid UK) and Mueller Hinton broth (Oxoid UK) were used.

0.5 McFarlan Standard: The inoculum was grown at 37^{0}C for 2-6 hrs. Turbidity Standard of 0.5 McFarland was achieved by incubating broth culture.

Inoculation of test plates:The plates were inoculated with the culture of *Acinetobacter* spp. by the help of sterile cotton swabs. The excess fluid was removed after the cotton swab was dipped into inoculum suspension. When the inoculum were dried the antibiotic discs were placed with sterile forceps onto the agar surface.[15]

Incubation of test plates: The isolates after application of antibiotic discs plates were incubated for 24 hours and results were interpreted according to CLSI standards [5,6]. Interpretative

standards for used antibiotics and Zone diameter of inhibition are shown in Table 2[5].

Control strain: *Escherichia coli* ATCC 25922was used as a control strain to maintain accuracy and precision of procedures.

Results

Table 1: Age and gender specific distribution of *Acinetobacter* spp. among patients

Age	**Male** n=32(61.5%)	**Female** n=20(38.46%)
00-25	10	06
26-50	05	02
51-75	12	11
76-100	05	01

Table 2: Zone diameter interpretive standards for *Acinetobacter* spp. CLSI standards table of antibiotics for *Acinetobacter spp.*

Antibiotic	**Disc Content**	**Zone of Inhibition (mm)**		
		Resistance	**Intermediate**	**Sensitive**
Amikacin	30µg	≤14	15-16	≥17
Cefoperazone	75 µg	≤15	16-20	≥21
Ceftriaxone	30 µg	≤13	14-20	≥21
Ciprofloxacin	5 µg	≤15	16-20	≥21
Colistin*	10µg	≤11		≥17
Fosfomycin*	50 µg	≤12	13-15	≥16
Imipenem	10 µg	≤13	14-15	≥16
Polymixin B*	300units	≤13		≥19

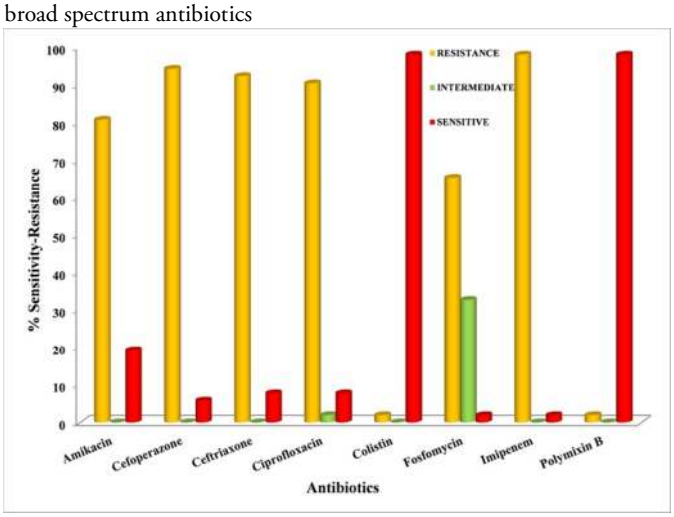
*Since the interpretive standards for Colistin, Fosfomycin and Polymixin B against *Acinetobacter* spp. is not established in CLSI 2013 mannual zone diameter interpretative standards for Enterobacter spp. and *E. coli* were used.[20]

Table 3: Total % efficacy of different antibiotics among *Acinetobacter* spp. isolated (N= 52)

Antibiotics	**Disc Code**	**Resistance (%)**	**Intermediate (%)**	**Sensitivity (%)**
Amikacin	30µg	42(80.76)	00	10(19.23)
Cefoperazone	75µg	49(94.23)	00	03(5.76)
Ceftriaxone	30µg	48(92.3)	00	04(7.69)
Ciprofloxacin	05µg	47(90.38)	01(1.9)	04(7.69)
Colistin	10µg	01(1.9)	00	51(98)
Fosfomycin	50µg	34(65.38)	17(32.69)	01(1.9)
Imipenem	10µg	51(98)	00	01(1.9)
Polymixin B	300 units	01(1.9)	00	51(98)

It is reported that out of all the samples 61.5% were obtained from male patients. Infections caused by *Acinetobacter*spp. had a high prevalence among both the genders among the age group 51-75 yrs. The most frequent site of isolate collection was tracheal aspirate (55.76%) among both genders and the second highest percentage of isolate was obtained from sputum (19.23%) as shown in Table 1. The Colistin and Polymixin B were found equally effective against*Acinetobacter* spp. by inhibiting 98% of isolates each and 19.23% isolates showed sensitivity against Amikacin. The isolate showed the highest degree of resistance against Imipenem (98%), followed by Cefoperazone (94.23%) and Ceftrioxone (92.3). Surprisingly 32.69% of isolates exhibited intermediate sensitivity (IS) against Fosfomycin as indicated in Table 3 and Figure 1.

Figure 1: Susceptibility pattern of *Acinetobacter* spp. against broad spectrum antibiotics



Discussion

Our present study shows that the *Acinetobacter* spp. were highly resistant to Cefoperazone (94.23%). This finding is further substantiated by research that observed Cefoperazone to be only effective when used in combination.[7,8]

We also observed that only 19% isolates were sensitive to Amikacin, which contradicts the findings of Liu et al 2013 [3]who observed 100% efficacy. However, they also discovered that 82% were inhibited by Imipenem while Fluoroquinolone was also found to be effective against 70% of all isolated organisms and Cefoperazone as least effective.

Organisms isolated from sputum showed a high degree of resistance to most of antibiotics, Zheng W and Yuan S also observed such results[9].Nwadike et al 2014[10] found a high prevalence of resistant *Acinetobacter* spp. isolates against Ciprofloxacin (100%) and Amikacin (50%).[10]

Polymixin inhibited 98% of isolates, which is similar to figures found by Haeili et al 2013[1] who observed 95.5% susceptibility to Polymixin B. The second most effective antibiotic was Colistin - Trottier et al 2007[12] also observed 100% susceptibility of *A. baumanni* to Colistin. Similarly, Vakilietal 2014[13] found a low rate (i.e, 11.6%) of Colistin resistance.

Colistin has emerged as a viable choice for treatment of multidrug resistant *Acinetobacter* strains. In several

studies,[13,14]where 98% of isolates were resistant to Imipenem these results support the work of Khajuria *et al* 2014[16] who also reported reduced efficacy. Our findings are in contradiction to the study by of Tripathi *et al* 2014[17] who reported that Imipenem was a highly effective drug in comparison to other broad spectrum antibiotics.Fosfomycin surprisingly exhibited unusual results in our study; 32% of *Acinetobacter* spp. were IS while 65% were resistant. However, previous studies showed that Fosfomycin were proved to be good option to treat infections caused by *Acinetobacter* spp.[18] Zhang *et al*2013[19] reported that Fosfomycin used alone was highly ineffective in treatment of Penicillin Drug Resistant-*Acinetobacter baumannii* (PDR-Ab).Another study revealed that *Acinetobacter* spp. has developed adaptive resistance against Polymixin.[21]

Acinetobacter spp. are emerging as a resistant bacteria and a common cause of nosocomial and hospital acquired infections. There is a serious need to take necessary measures by hospital administration in maintaining environmental and personnel cleanliness according to current Good Manufacturing Practices. Pharmacists should educate patients about the drawbacks of self-medication and not completing medication courses, which is resulting in development of resistant bacterial pathogens.

Competing Interests
None declared
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