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COLISTIN RESISTANCE IN *ACINETOBACTER LWOFFII*

ABHISEK ROUTRAY*, ANU M.S AND RADHA MADHAVAN

SRM Medical College Hospital and Research Center
Potheri, Kattankulathur, Kanchipuram, Tamil Nadu

ABSTRACT

Acinetobacter spp. has emerged as a significant hospital pathogen, quickly becoming resistant to commonly used routine drugs. Colistin remains as the only drug of choice for carbapenem resistant *Acinetobacter*. In our study among 65 strains of Acinetobacter isolated from different clinical samples, 2 strains showed Colistin resistance by Disk diffusion method .It was confirmed by performing minimum inhibitory concentration (MIC) using E-strip. To assess the virulence factor biofilm production was also detected in all strains, the 2 strains which showed extreme drug resistance (being colistin resistant) was also strong biofilm producers. Such strains showing extreme drug resistance and strong biofilm production remain as a great threat.

KEYWORDS: ***Acinetobacter*, Colistin, biofilm, drug resistance.**

ABHISEK ROUTRAY
Srm Medical College Hospital And Research Center Potheri, Kattankulathur, Kanchipuram,Tamil Nadu

Corresponding author

INTRODUCTION

Acinetobacter species are aerobic gram-negative coccobacilli that have emerged as important opportunistic pathogen, especially among critically ill patients. The nosocomial infections caused by *Acinetobacter* species include bacteremia, meningitis, pneumonia, and wound infection. Because of frequent resistance to the aminoglycoside, fluoroquinolone and third-generation cephalosporin, carbapenem are important agents for managing acinetobacter infections [1,2]. Initial concern about multidrug-resistant and carbapenem-resistant *Acinetobacter baumannii* (CRAB)-associated infections began when the first hospital wide outbreak occurred in New York city in 1991. Since then, reports of CRAB have been accumulating from other parts of the world. [4,5]. Many of these strains remain susceptible only to colistin, a toxic peptide antibiotic. The rapid evolution of drug resistance has severely limited the options for effective therapy for infections caused by this pathogen. Colistin resistant *Acinetobacter* is also now emerging and is a major threat greatly limiting the therapeutic option.

MATERIALS AND METHODS

Identification of the organism

Strains of *Acinetobacter* were collected from routine clinical samples. Identification was done by routine biochemical tests including Gram staining , oxidase , catalase , motility test Indole , Methyl red , Voges Prousker, citrate , Triple sugar iron agar , Urease test , mannitol motility medium and growth on Mac-conkey agar , blood agar . Speciation of *Acinetobacter* was done using an oxidation fermentation test and utilization of carbon source such as DL-4-amino butyric acid, L-Phenyl alanine, L-Phenyl acetate, DL-Aspartic acid, L-Thyrosine.

Antibiotic susceptibility testing

Antimicrobial susceptibility tests were performed by Kirby Bauer disk diffusion method as per CLSI guidelines. All the routine drugs were tested including Colistin drug (10µg).

Minimum inhibitory concentration for colistin using E-strip(Colistin Ezy MIC^{TM} Strip HIMEDIA):

Mueller Hinton agar Plates were swabbed with 4 hrs culture of the Isolate grown in Nutrient broth.E strips were placed with sterile stick. Incubated for 24 hrs and reading was taken.

Interpretation : **(as per CLSI guidelines)**

Resistant: ≤2µg/ml

Sensitive: ≥4µg/ml

Detection of bioflim production

(Using microtiter plate method)

The strains were freshly sub-cultured on nutrient agar plates. The sub-cultured strains were inoculated in Trypticase Soy Broth (TSB) and incubated at 37^{o} C for 24 hours. In a microtitre plate 230µl of TSB was added followed by 20µl of the inoculated broth .3 wells were used for each strain i.e. Blank, Positive control, Negative control. The micro titre plates were incubated at 37^{o} C for 24 hours. [13]

Washing of the microtitre plate

Wells were washed with Phosphate Buffer Saline (PBS) thrice which contains Nacl-9gm, Sodium dihydrogen phosphate -1.5gm and Di sodium phosphate 5.76gm in 1000 ml of distilled water]. Fixation was done with methanol. Wells were washed with distilled water and 250µl of crystal violet [0.1%] was used to stain the well and kept on shaker for 15 minutes followed by washing with sterile distilled water. Then 250µl of Glacial acetic acid [33%] was added and kept on a shaker for 15 minutes. It was then read using a ELISA reader at 450 nm.

Interpretation

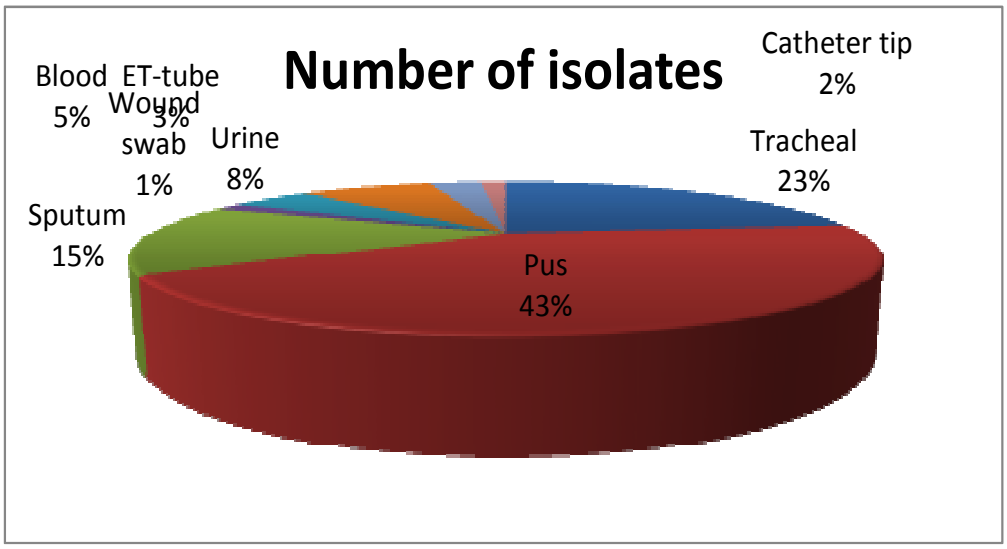
The classifications were: no biofilm formation OD≤ODc; weak biofilm formation 2×ODc≥OD>ODc; moderate biofilm formation

4×ODc≥OD>2×ODc; and strong biofilm formation OD>4×ODc, according to Stepanovic *et al*. (2004).

RESULTS

A total of 65 strains of *Acinetobacter* were collected from various clinical samples

Sample	Number of isolates
Tracheal	15
Pus	28
Sputum	10
Wound swab	1
Blood	3
Urine	5
ET-tube	2
Catheter tip	1



Out of 65 strains of Acinetobacter, 46(70.7%) were *Acinetobacter baumannii* and 19(29.2%) were *Acinetobacter lwoffii.* High resistance was noted for Penicillin (Ampicillin-94% followed by Cephalosporin(Cefepime - 83.07%),Aminoglycosides (Amikacin - 76.3%,Gentamycin- 81.53%) and Fluoroquinolones (Ciprofloxacin-78.46%) Carbapenem (Imipenem-64.61%) and Polymyxin (Colistin-3.1%) . Minimum Inhibitory Concentration (MIC) was performed for Colistin using E strip, for the 2 strains which showed resistance by disk diffusion method. It showed a MIC of 6 and 12 µg/ml respectively (figure-1)

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Figure 1
E test for colistin

Biofilm production

Out of the 65 strains of *Acinetobacter*, 7 were strong Biofilm producers, 18 were moderate biofilm producers , 20 were weak biofilm producers and 20 were non biofilm producers . Both the strains which were Colistin resistant were A.lwoffii, they showed resistance to all the routinely used drugs and were strong biofilm producers (figure-2)

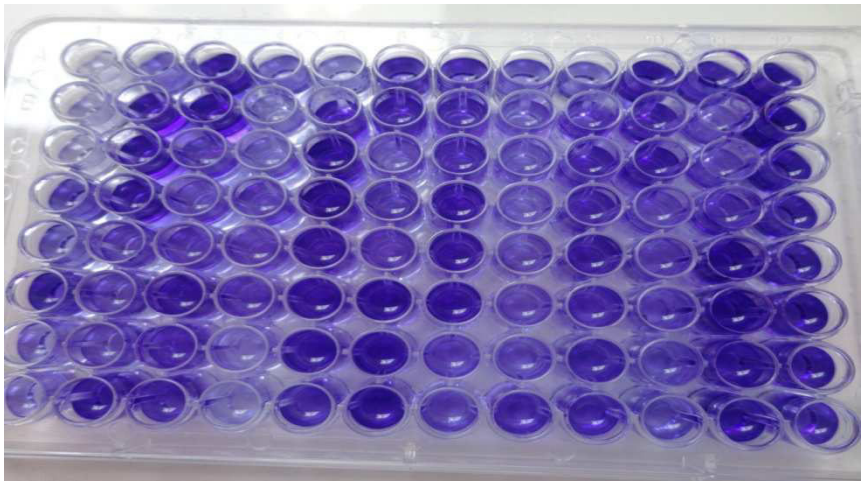

Figure 2
Biofilm production by Microtiter plate method.

DISCUSSION

Due to the recent emergence and worldwide spread of multidrug-resistant *Acinetobacter* only few antimicrobial drugs remain available for effective treatment. Colistin is the last resort for treatment of multidrug-resistant *Acinetobacter baumannii*. Unfortunately, resistance to colistin has been reported all over the world. The highest resistance rate was reported in Asia, followed by Europe. The heteroresistance rate of *A. baumannii* to colistin is generally higher than the

resistance rate. The mechanism of resistance might be loss of lipopolysaccharide[3]. Pharmacokinetic/pharmacodynamic studies revealed that colistin monotherapy is unable to prevent resistance, and combination therapy might be the best antimicrobial strategy against colistin-resistant *A. baumannii* [9]. Emergence of such extreme resistance greatly limits the therapeutic use. The clinical significance of colistin-resistant strains has recently been highlighted with the report of the emergence of colistin resistance after Colistin treatment of an infection caused by a heteroresistant *A. baumannii* strain (10). In our study a resistance rate of 3.1% was noted by *A.lwoffii* by using E-strip. According to one study, sensitivity of the E-test was 90.9%, and its specificity was 100%; the positive and negative predictive values were 100 and 97.8%, respectively. [12]

Colistin has already been reported as an adequate alternative in sporadic cases of nosocomial infections by multidrug-resistant *A.baumannii* [9]. Since the panel of its adverse effects limits its application only for infections by multidrug-resistant isolates. Studies demonstrated significant association of biofilm with multi drug resistance. Bioflim production in *A.baumannii* might promote increased colonization and persistence leading to higher rates of device related infections. [5] Leriche et al. found that exposure to antimicrobial agents induced the cells in the biofilm to coexist in mixed structures, suggestive of protection by the more resistant species of other members of the biofilm. [11] .Our studies also showed significant increase in drug resistance with respect to biofilm production . The two strains which showed colistin resistance were strong biofilm producers . Reports regarding *A.lwoffii* are less, so importance must be given for speciation in routine diagnosis.

CONCLUSION

Extreme drug resistance encountered in clinical environment posses a great treat to patients as coistin remains as the only drug of choice for multi drug resistant *Acinetobacter* infection. Production of biofilm adds to the virulence factor, which makes the treatment option even more complex.. Hence judicious use of colistin drug is highly recommended.

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