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Surveillance of *Pseudomonas aeruginosa* with colistin resistant at ICU of a tertiary care hospital

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Abstract
Background: The gram-negative bacteria; *P aeruginosa* is often a motive of nosocomial infections like; meningitis, bacteremia, pneumonia, and urinary tract infections. *P aeruginosa* is known as an emerging motive of nosocomial infections worldwide. Colistin is the ultimate line treatment for multidrug-resistant A. Baumannii, and unfortunately, its resistance to colistin is being suggested from all around the world.
Materials and Method: This prospective observe became carried out at some point of Jeb 2016 to Jan 2018 (2 years) on one hundred P aeruginosa samples remoted from Wound swabs, urine, respiratory secretions, and blood of sufferers admitted to the intensive care unit of IMS and SUM Hospital, Bhubaneswarm India. The micro-organism were identified through microscopy and biochemical tests. The disk diffusion method with colistin disk (Himedia, Mumbai, India) became utilized to discover the resistance to colistin.
Results: A total of a hundred P aeruginosa microorganism have been identified, of which 93 had been sensitive and seven were proof against colistin, respectively, consistent with the disk diffusion test.
Conclusion: The outcomes confirmed that the resistance of P aeruginosa to colistin, as the final line remedy, is rising in Bhubaneswar, Odisha, India.

Keywords: *P aeruginosa*, disk diffusion, multidrug resistance, Colistin

1. Introduction
P aeruginosa is a Gram negative, non-fermenting, opportunistic isolate, that is recognized as a major nosocomial pathogen. It can cause infections at various anatomical sites; bacteremia, pneumonia, meningitis and urinary tract infection, most commonly in immunocompromised and critical care patients. The capacity to endure on dry surfaces and its relative resistance to disinfectants allows this non-fermenter to survive well in the hospital environment [1]. *P aeruginosa*isolates are resistant to almost all available antibiotics including β-lactams, fluoroquinolones, tetracyclines, aminoglycosides and carbapenems [2]. More importantly, pandrug-resistant and extremely drug-resistant isolates have emerged [3] and are on the rise worldwide. Colistin (polymyxin E) and tigecycline are frequently the only antibiotics remaining to treat multidrug resistant (MDR) *P aeruginosa* infections [4]. However, hetero-resistance and resistance against colistin have been reported in clinical settings throughout the world [5]. Here we review the reports all over the world and epidemiology of colistin resistance in *A. baumannii*. Resistance against Colistin Colistin, a natural substance produced by Bacillus polymyxa and a cationic lipopeptide (cyclic decapeptide) discovered in 1949. It has not typically been included in regimens to treat Acinetobacter infections because (Albeit debatable) of its neurotoxicity and nephrotoxicity. However, it has been as a therapeutic option for the treatment of ventilator associated pneumonia caused by drug resistant gramnegative organism [6]. Colistimethate sodium and Colistin sulfate are two commercially available forms and recently colistin has progressively been used as rescue therapy for severe infections in critically ill patients [7]. It is bactericidal against Gram negative bacteria; its amphiphilic nature allows it to interact with lipid A moiety of lipopolysaccharide (LPS) causing disarray in the bacterial outermembrane. Colistin is consists of cyclic heptapeptide covalently attached to a fatty acyl chain [8]. Typically colistin resistance is by chromosomally mediated modulations. There is relatively little research has been done on colistin resistance in *P aeruginosa*and there are two main hypotheses of colistin resistance.

The first hypothesis of colistin resistance is mediated by loss of LPS production, caused by mutations in any of lipid A biosynthesis genes (lpxA, lpxC and lpxD) terminating complete production of LPS. Furthermore, presence of insertion sequence ISA ba11 in either lpxC or lpxA not only causes loss of LPS production but also causes high level colistin resistance [9]. In countering to total LPS loss, *P aeruginosa* modify the expression of critical transport and biosynthesis systems associated with modulating the composition and structure of the bacterial surface. Eventually, LPS deficiency causes less negative charge and thus might be the reason for the loss of affinity towards colistin [10]. Secondly, colistin resistance has been hypothesized to PmrAB-two component response regulator and sensor kinase system. This system allows bacteria to sense and respond to various environmental conditions such as pH or Fe3+ and Mg2+ levels, also affecting expression of genes implicated in lipid A modification and thus causing colistin resistance [11]. Point mutations in pmrA and pmrB genes of PmrAB two-component regulatory system showed upregulated expression of pmrAB. The increase expression results in remodeling of bacterial membrane causing decreased membrane permeability [12]. Recently, colistin resistance has shown to be singularly due to plasmid mediated mcr-1 gene. Although there is no report of mcr-1 being detected in *A. baumannii,* the prevalence has been investigated in E. coli and K. pneumoniae [13, 14]. If mcr-1 gene is similar to NDM-1 colistin resistance could become endemic in the world. The rapid dissemination of previous antibiotic resistance indicates that, with the advent of transmissible colistin resistance, progression of *P aeruginosa* from multidrug to pandrug resistance is unavoidable. Although the levels of maximum inhibitory concentration of colistin are not high (4–8 mg/L), acquaintance of mcr-1 by carbapenem resistance *P aeruginosa* isolates will make them resistance to all antibiotics [14]. The potential of mcr-1 to become global depend upon several factors: use of irrational doses of colistin, the stability of mcr-1 mediated plasmid and their ability to transfer in humans. Effective strategies that limit selection and further dissemination of plasmid associated mcr-1 are clearly needed. It is important to prevent the dissemination of colistin by developing agents which provide effective reverse resistance strategies. Lately, colistin resistance has been found due to efflux pumps [15] in which efflux pump inhibitors (EPIs) were used to suppressed colistin resistance. Colistin resistance has been attributed to efflux pumps belonging to RND (resistance-nodulation-cell division) family [16]. The efflux pump consists of two component regulatory system mediating adaptive response of bacterial cells to a range of environmental stimuli. Genes are organized as operon adeA, adeB, and adeC and regulated by adeR gene. adeA is a membrane fusion protein and adeC is an outer membrane protein channel, in which adeB acquire its substrate and transports from cytoplasm or within phospholipid bilayer to extracellular medium [17].

In this study we have found the incidence of constin resistance on *P aeruginosa* from a tertiary care teaching hospital.

2. Material and Methods

This study was carried out during 24 months in the intensive care unit of IMS and SUM Hospital, Bhubaneswar Hospital in Odisha. Sampling was performed from Wound swabs, ulcers, blood, and respiratory secretions of patients admitted to ICU. The bacteria were cultured in Eosin Methylene Blue, blood agar, and MacConkey's agar media. After inoculation, the samples were placed on the relevant culture medium. The cultured plates were then incubated at 37 °C for 18-24 hours. *P aeruginosa* (Fig 1) was recognized using the tests catalase, oxidase, TSI, and motility; other biochemical tests were performed and antibiogram (Fig 2) was performed on 100 purified samples through the following general procedure. To prepare a suspension of *P aeruginosa* with turbidity equal to that of half McFarland, a number of *P aeruginosa* colonies were dissolved in some sterile normal saline to produce a suspension with half McFarland turbidity; a swab was entered in the suspension and its extra solution was squeezed, the swab was then streaked slowly in different directions on Mueller Hinton agar medium so that its entire surface was covered uniformly with the bacteria; after the surface was dried (not to be more than a quarter), antibiotics disks (Himedia, Mumbai) were placed on the medium at least 2 cm apart from each other using sterile forceps; the plates were then incubated at 35 °C for 18-24 hours. Finally, the zone of inhibition was measured with a ruler and matched with CLSI tables for different antibiotics.

3. Results

All 100 isolates identified as *P aeruginosa* in this study had the same biochemical pattern. *P aeruginosa* produced pale pink mucoid colonies on blood agar (without hemolysis and pigment) and MacConkey's agar media. At the end of this study, 100 strains of *P aeruginosa* were recovered in IMS and SUM Hospital, Bhubaneswar Hospital using microscopy and biochemical tests and by eliminating unrelated and repeated isolates. Most isolates were isolated from Wound swabs (Table 1). A total 93 *P aeruginosa* isolates sensitive to colistin *and* 7 were resistant were documented in IMS and SUM Hospital, Bhubaneswar.

Table 1: Percentage of distribution of *P aeruginosa* isolates based on clinical samples

Sample	***Number***
Blood	18
Tracheal aspiration	11
ulcer	26
Wound swab	45
Total	100

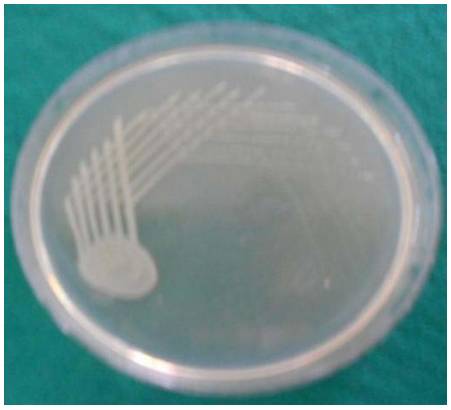

Fig 1: Pure cultre of *P aeruginosaon nutrient agar*

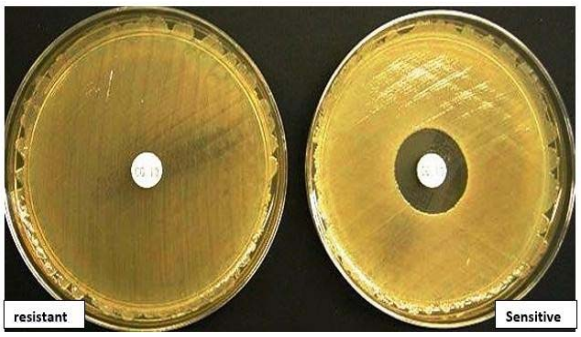

Fig 2: Colistin resistance and sensitive

4. Discussion

High resistance of *P aeruginosa* to different antibiotics and prevalence of multidrug-resistant strains (MDR), especially in immunosuppressed patients, makes difficult the control and treatment of the disease. Infections caused by this microorganism have a negative impact on clinical outcomes and treatment costs. As a nosocomial- inducing bacterium, *P aeruginosa* creates many health problems in hospitals in Iran like the rest of the world; therefore, knowledge of its resistance to colistin is of great importance in the treatment of *A. baumannii*-induced infections. In comparison with similar studies in other regions of Iran, the frequency of colistin-resistant isolates of *P aeruginosa* was high in this study. The difference in these findings may represent the increasing resistance of these strains to colistin, so that comparison of the studies dates confirms this increment over time. In this research, 6% of the isolates were resistant to colistin which was the greatest resistance rate compared with other studies. Reports are increasing after the first report of colistin-resistant Acinetobacter spp. in 1999 in the Czech Republic [7]. Li *et al*. described colistin-resistant *P aeruginosa* for the first time in 2006 [8]. The resistant of *P aeruginosa* to colistin in clinical isolates is a serious danger, suggesting that if colistin is inappropriately used, a rapid increase in resistance and treatment failure are likely to occur. The antimicrobial surveillance department including the United States of America, Europe, Latin America, and Asia-Pacific region indicated that the resistance of *P aeruginosa* to colistin remained at a low level from 2001 to 2011 (0.9%-3.3%) [9- 11]. Other reports of colistin-resistant *P aeruginosa* were obtained from Asia, Europe, North America, and South America. Ten reports from Europe, including 2 case reports, provided information about colistin-resistant *P aeruginosa* [12-13]. The rate was less than 7% in most of these reports; [14], however the rates of two reports from Bulgaria and Spain were 16.7% and 19.1%, respectively [15-16]. Interestingly, this rate was 40.7% in another report from Spain in which the cases were recruited from a three-care hospital between 2000 and November 2006. According to [15], the rate in 7 out of 8 reports from Asia was less than 12%. Ko *et al*. reported the highest rate of colistin resistance as 30.6% in Korea [17-20]. Out of 3 reports from the United State [21], 2 had a relatively low resistance not more than 1.2% [22, 23]. The rate of resistance was not more than 1.7% in South America [24]. Asia and Europe have the most serious situation in terms of resistance to colistin, and have more reports and higher resistance rates, while North and South America had a lower rate of colistin-resistant *P aeruginosa* and fewer reports in this regard. Comparison of the results of this research with similar studies indicates increasing prevalence of nosocomial infections caused by colistin-resistant *A. baumannii*. According to the result obtained in this study and other regions of the world, the isolates of *P aeruginosa* resistant to colistin are increasing in Iran, and infections caused by these microorganisms will adversely affect clinical outcomes and treatment costs. Spread of colistin-resistant *P aeruginosa* in Iran is similar to Korea in Asia and shows that this colistin-resistant organism is increasing in Asia. Since no new drug is on the way to replace the existing drugs against Gramnegative pathogens, and no wide coverage vaccine exists against these infections, the only way to mitigate the spread of infection is to control the bacteria prevalence. This is only achievable by completely understanding the causes, dynamics, and complexity of the occurrence of these organisms.

5. Conclusion

This research isolated colistin-resistant *P aeruginosa* strains from hospitals in Bhubaneswar; this necessitates adopting appropriate treatment regimen and application of detailed strategies for prevention of nosocomial infections caused by these bacteria.

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