Prevalence of Multi-drug Resistant (mdr) *Pseudomonas sp.* Among Patients Attending in Tertiary Care Hospital, Dhaka City, Bangladesh

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Abstract

The resistance pattern of Pseudomonas sp. isolates in recent years is increasing very rapidly because of the uncontrolled, unsupervised, and unnecessary usage of antibiotics. Current study aims to evaluate the prevalence of multidrug-resistant (MDR) Pseudomonas sp. isolated from clinical samples collected from tertiary care hospitals in Dhaka, Bangladesh. In this study, a total of 1440 samples were collected from January 2019 to September 2019, followed by using conventional methods, as well as the Kirby-Bauer disc diffusion method for culture and susceptibility of Samples, respectively. Finally, Stata 14 software was utilized to analyze the obtained data. From among 1440 samples, 480 (33.3%) cases were bacteriologically positive. Imipenem (11%) showed the least resistance followed by Meropenem (20%), where Nalidixic acid exhibited the highest drug resistance. Additionally, 202 (48.08%) and 278 (57.92%) cases were identified as MDR and non-drug-resistance (NDR). The number of male MDR cases was higher than females. It is alarming to increase MDR pathogens. This bacterial resistance can be prevented through control measures that limit the spread of resistant bacteria and the regular monitoring of this resistance phenotype of pathogens, along with the rational use of antimicrobial therapy.

Keywords: Multidrug-resistant (MDR), Pseudomonas sp., Kirby-Bauer disc diffusion, Tertiary care hospital, and Dhaka city.

Significance | Unchecked antibiotic use is making Pseudomonas sp. more resistant. To combat this, it's crucial to manage antibiotics responsibly.

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Introduction

Pseudomonas sp is a leading cause of nosocomial infections. In developed countries of Western Europe annually four hundred thousand cases of hospital-acquired infections are reported among which a third are Pseudomonas sp. (Adeleye & Adetosoye, 1993; Aguilera-Sáez et al., 2019). Infections caused by Pseudomonas sp are often severe and life-threatening and are difficult to treat because of the limited susceptibility to antimicrobial agents and the high frequency of emergence of antibiotic resistance during therapy (Algammal et al., 2020; Anthony et al., 2002; Asma et al., 2019) thus resulting in severe adverse outcomes (Baban, 2020; Berrouane et al., 2000; Bhuiya et al., 2018).

Pseudomonas sp is a clinically important gram-negative bacterium, which is responsible for a variety of systemic infections like urinary tract infections, respiratory system infections, gastrointestinal infections, dermatitis, bacteremia, soft tissue infections, bone and joint infections (Bronzwaer et al., 2002). Due to its nominal nutritional requirement, Pseudomonas sp can survive in soil, plant surfaces, wastewater, moist environment, surface water, or even on inert materials (Brun-Buisson et al., 1987). Using life support equipment, cosmetics, dilute antiseptics even through the washing liquids and soaps, it can be transmitted from person to person (Carmeli et al., 1999; Chaoui et al., 2019). However, Pseudomonas

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sp. is mostly acquired from the environment and spread person-to-person rarely (Clinical and Laboratory Standards Institute [CLSI], 2013). The problem of antibiotic resistance in Pseudomonas sp. is on the increase (Aguilera-Sáez et al., 2019). The high frequency of drug resistance results from newly emerging resistance in specific organisms after exposure to antibiotics (Algammal et al., 2020) and patient-to-patient spread of resistant organisms (Berrouane et al., 2000). Resistance after exposure to antibiotics may result in multidrug-resistant (MDR) Pseudomonas sp. Pseudomonas sp is highly ubiquitous in water systems and capable to acquire antibiotic resistance due to its low outer membrane permeability and extensive efflux pump system (Devi et al., 2020; Dimopoulos et al., 2020). The overall prevalence of antibiotic-resistant Pseudomonas sp is increasing, with up to 10% of global isolates found to be multidrug resistance (Gales et al., 2001). It is recognized as the second leading cause of gram-negative nosocomial infection and a major treatment challenge for Pseudomonas sp (Hauser et al., 2002; Jameson et al., 2018).

There are a huge number of hospitals and clinics in Dhaka City due to being a densely populated city, where antibiotics are used frequently. The use of antibiotics is included but is not limited to medicine, veterinary medicine, and growth promoters in animal husbandry, fish farming, etc. After use antibiotics are often released into the water. Frequent use of antibiotics in treatments plays an important role in the emergence of resistant bacteria (Kato et al., 2001; Khan et al., 2020). Antibiotic producing pharmaceuticals are located in areas that are close to water reservoirs and very few of them maintain the guidelines to dispose of effluents in ponds, rivers, and other water systems.

The presence of antibiotic-resistant microbes has been observed in wastewater, surface water, groundwater, sediments, and soils. With changing and increasing resistance observed in clinically significant bacteria, it is important to detect drug-resistant microorganisms. In the past two decades, the emergence of resistance to antimicrobial agents has been repented as one of the most important clinical crises (Kugelberg et al., 2005; Kutty, 2011). Because of variety among beta-lactamases, alterations in binding proteins and outer membrane permeability, and multiple mechanisms of resistance, the gram-negative bacteria have acquired resistance against extended-spectrum Cephalosporins, Monobactams, and Carbapenems and (Lai et al., 2018). Extended spectrum beta-lactamases (ESBLs) are an important reason for bacterial resistance throughout the world (Law et al., 2019). As ESBL producing isolates can hydrolyze 3rd and 4th generation Cephalosporins and Mono-bactams Microbiologists, clinicians, infection control professionals, and scientists are hard-pressed to find new antimicrobial agents. ESBL producing strains are only inhibited by beta-lactamase inhibitors, clavulanic acid, sulbactam, and tazobactam (Liao et al., 2019; Lister et al., 2009; Moradali et al.,
2017).

Materials and Methods

Isolation of Pseudomonas spp.

Blood Samples were collected and transported to the Department of Microbiology, Primeasia University at the earliest convenience, and samples were inoculated directly onto MacConkey's, blood, and Cetrimide agar plates in triplicate. Cetrimide agar was used for selective isolation of Pseudomonas sp.. The plates were then incubated overnight at 37°C in an incubator (Binder, Germany) and examined for non-lactose fermenting pale colonies (Nathwani et al, 2014).

Identification of Pseudomonas spp.

Morphological characteristics & microscopy

Gram-negative rods were expected in gram staining. The organism on the MacConkey agar plate produced non-lactose fermenting transparent greenish colonies (Nathwani et al, 2014) Green color colonies were observed on cetrimide agar.

Biochemical test

The isolated Pseudomonas spp. were further confirmed by biochemical tests following the procedure by using biochemical tests like Triple Sugar Iron (TSI-Oxoid), Motility Indole Urea (MIU-Oxoid), and Simmons Citrate (Oxoid) agar [29]. Kovac's reagent (LOBA Chem, Italy) was used to detect tryptophan hydrolysis for the Indole test. In comparison to other modern and rapid tests, biochemical tests were selected as a method of identifying isolates due to their availability and cost-effectiveness for the current study.

Determination of antimicrobial susceptibility

Bacterial susceptibility to antimicrobial agents was determined by using the Kirby-Bauer disc-diffusion method and Clinical and Laboratory Standard Institute guidelines (CLSI), by using antibiotic-containing disks from Oxoid Ltd, UK as well as the manufacturer instructions about the clear zones of growth inhibition around the disks (Paghdar et al, 2020; Palavutitotai et al, 2018)

Statistical analysis

The chi-square test was utilized to compare the groups and $P<0.05$ was considered as statistically significant which was determined using Microsoft excel 2007 (NNIS, 1992).

Results and Discussion

Isolation and identification of Pseudomonas spp.

Out of 1440 samples, 480 positive samples (30%) were isolated and later identified as Pseudomonas sp. based on their morphological characteristics and biochemical characteristics (Ray et al, 2010).

Determination of antimicrobial susceptibility

Results from antimicrobial activity revealed that Imipenem is the best drug for treatment against Pseudomonas sp. based on the current study, followed by Meropenem. However, other generation cephalosporin drugs showed higher resistance in comparison to Ceftazidime. Cotrimoxazole also exhibited satisfactory results based on antibiograms (Ray et al, 2010).

Statistical analysis

Statistical analysis was conducted as ANOVA, which revealed a significant value with P-value < 0.05 (0.008) (Roy-Burman et al, 2001).

To study the prevalence of multidrug-resistant of Pseudomonas sp, a total of 1440 Pseudomonas sp infection suspected population was diagnosed but 480 samples were found Pseudomonas sp. positive. Fattma et. al. showed a 13.88% positive culture among 144 total samples (Nathwani et al, 2014). A recent study found 30% positive for a urine sample, the most common source of isolation. However, our study has not categorized the different samples but has found a similar finding, of a 30% positive prevalence of Pseudomonas sp. (Ray et al, 2010).

The emergence of MDR Pseudomonas sp (resistant to Cephalexin, Cephearadine, and co-trimoxazole) initiated, consistent use of fluoroquinolones that had proven to be effective for the treatment of Pseudomonas infection. This study has been conducted to observe the prevalence and antibiotic susceptibility pattern of Pseudomonas sp in Dhaka city (Ray et al, 2010).

It was seen that 11% (Imipenem) to 57% (Nalidixic Acid) of the Pseudomonas sp. strains are resistant to the antibiotics used against them. Imipenem (89 % sensitive) is the best drug for the treatment of Pseudomonas sp. infection followed by Meropenem (70% sensitive) where 10% of meropenem isolates were moderately sensitive. The results of Fattma et.al showed that the most effective antibiotics were Pip/Tazo and Imipenem (80% sensitivity), followed by Meropenem and Amikacin (75% sensitivity) (Nathwani et al, 2014).. That result is similar to findings to the current study in the case of Imipenem and Meropenem. Shi et. al showed that 41.8 and 25.6% of patients were admitted to the ICU due to Pseudomonas auroginosa infection as well as exhibiting hospitalized patients having a record of prior exposure to antibiotics or immunological system defects, which leads to resistance against antibiotics. Therefore, this may contribute to the reason of other drugs being resistant, in the current study compared to that of a previous study (Seppänen et al, 2008). In a total of 480 samples, 61 cases (76.25%) and 38 cases (47.50%) were reported positive for multidrug-resistant in females above and below 30 years of age respectively.

In the case of Multidrug resistance sixty-one out of eighty samples (76.25%) were multidrug-resistant found in the sample of elder females, thirty-eight among eighty samples (47.50%) were multidrug-resistant found in the sample of young females, forty-four out of eighty samples (55%) were multidrug-resistant found in the sample of young male & fifty-nine samples (73.75%) were multidrug-resistant found in the sample of elder males. Furthermore, 202 of the samples (42.08%) were resistant to at least 5 antibiotics. In a conducted study it was found that 56 (22%) strains were XDR-PA, 32 (12.5%) were MDR-PA, 167 (65.5%) were non-MDR PA, and 0 were PDR-PA strains out of a total of 255 patients (Shi et al, 2019; Tang et al, 2018). A meta-analysis conducted had reported that all isolates in two studies were completely MDR (100%). While in another study it showed, 44.4% and 18.2% MDR and XDR respectively among Pseudomonas auroginosa isolates (Uddin et al, 2018) which has a higher relevance in findings compared to that of other findings which exhibited lower drug-resistant pattern among isolates (Uddin et al, 2018; Vandewoude et al, 2000).

Conclusion

In developing countries, the prevalence of Pseudomonas sp. is alarmingly high whereas, in developed countries the incidence of Pseudomonas sp. is rare comparatively. Furthermore, the findings conclude that the main concern of the high infection is the multi-drug resistant phenomenon which is identified in commonly used antibiotics. However, current studies use the antibiotic, Imipenem as a preferred drug for testing sensitivity but in this study isolates of Pseudomonas sp. have displayed a significant 11% resistance. In patients with an immunocompromised system, the infection will transmit faster resulting in diseases like pneumonia, endocarditis, peritonitis, meningitis, ecthyma gangrenosum (EG), bacteremia, and septicemia.

Author contributions

S.D., conceptualized and developed the methodology, S.M.K.I., and M.A., prepared the original draft and collected, A.N.F., reviewed and edited the writing.

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Competing financial interests

The authors have no conflict of interest.

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