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Dissemination of Multidrug-Resistant, Extensively Drug Resistant and Pandrug-Resistant *Pseudomonas aeruginosa* Isolates among In-Patients and Out-Patients in a Multi-Profile Health Care Settings

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Pseudomonas aeruginosa is one of the most life-threatening pathogens, especially in healthcare settings, and a main contributor to multi-drug resistance (MDR), extensive-drug resistance (XDR), and pan-drug resistant (PDR) phenotypes. However, there is limited data on the degree of resistance of these isolates in this region. This study seeks to determine the distribution of MDR, XDR, and PDR *Pseudomonas aeruginosa* strains from different patient groups. A total of five hundred (500) non-duplicated strains of *Pseudomonas aeruginosa* of human clinical samples were collected from the Microbiology Laboratory Unit of Alex Ekwueme Teaching Hospital Abakaliki. The isolates were identified and re-characterized by standard microbiology techniques. MDR, XDR, and PDR were determined using the Kirby–Bauer disk diffusion method, and the results were analyzed using the Clinical Laboratory Standard Institute (CLSI) zone diameter breakpoints. The result shows that the overall resistant phenotype was MDR 50. 7%, XDR 20.5%, and PDR 9.6% while in samples from in-patients and out-patients, resistant phenotype proportions were MDR 43.2%, XDR 32.4%, PDR 10.1% and MDR 61.2%, XDR 29.7%, and PDR 18.5% respectively. Worrisomely, only a few tested antimicrobial agents (Amikacin, cefepime) were active against the test organism, presenting a limited therapeutic option. It is therefore imperative to establish strong regulative measures and guidelines that would help in curtailing the increasing dissemination of these superbugs in healthcare institutions in Nigeria.

Keywords: Multidrug-resistant; extensively drug resistant; pandrug-resistant; Pseudomonas aeruginosa.

1. INTRODUCTION

Pseudomonas aeruginosa is one of the most important causes of community and healthcare-related opportunistic infections among Gram-negative bacteria [1]. *P. aeruginosa* infections are very difficult to eradicate due to their intrinsic resistance to antibiotics, in addition, to various virulence factors like flagellin and lipopolysaccharide, as well as secreted products such as cytotoxins, elastase, alkaline protease, protease IV, as well as its invasiveness and increased colonization has been reported to contribute to its pathogenicity [2]. *P. aeruginosa* is divided into different phenotypes based on the drug resistance patterns of the organism. Multidrug‑resistant (MDR) phenotype is defined as *P. aeruginosa*, which is resistant to more than one antimicrobial agent in three or more antimicrobial categories. A similar resistance to more than one antimicrobial agent in <3 antimicrobial categories are defined as drug‑resistant (DR) *P. aeruginosa*. Extensively DR (XDR) phenotype is defined as *P. aeruginosa*, which is resistant to more than one antimicrobial agent in all the antimicrobial categories, except in two or less. Pan‑drug (PDR) phenotype is defined as a bacterium which is resistant to all antimicrobial agents in all antimicrobial categories [3,4]. Worldwide *Pseudomonas aeruginosa* is one of the most common life threatening pathogens, and a main contributors to multi-drug resistance (MDR), extensive-drug resistance (XDR) and pan-drug resistant (PDR) phenotype in hospital and community settings [5-10]. MDR, XDR and PDR strain are capable of stalling the action of antimicrobial agent due to the production of enzyme such as extended‑spectrum beta‑lactamases (ESBL) and metallo β‑lactamases (MBL), that truncate the action of beta‑lactams and carbapenems [1,11-13]. The dissemination of MDR, XDR and PDR through patients' movement from community to hospital or vice-versa, may facilitate the spread and convergence of such resistance phenotype among inpatients and outpatients. With the ever-increasing level of indiscriminate use of antimicrobial agents and rapid dissemination of MDR, XDR and PDR strains in the tropical countries like ours, the best therapeutic approach has proved to be controversial, leaving few alternatives for treatment of these patients. The menace of MDR, XDR and PDR *Pseudomonas aeruginosa* is of physical, emotional and financial detriment to patients globally with increased morbidity and mortality. Therefore, the need to identify and ascertain the rate of MDR, XDR and PDR *Pseudomonas aeruginosa* in a multipurpose healthcare system is of public health importance.

2. METHODS

2.1 Study Area and Ethical Consideration

This study was carried out at Alex Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA), Ebonyi State. It is a multipurpose healthcare setting in Abakaliki town, the capital city of Ebonyi State and it's located at 6.3231°N latitude and 8.1121°E longitude. The ethical approval for the study was granted in 2022 by the AE-FUTHA Ethics and Research Committee with reference number: SMOH/ERC/043/22. Every fundamental study was done in accordance with the ARRIVE guidelines.

2.2 Bacteriological Analysis

A total of five hundred (500) non-duplicated *Pseudomonas aeruginosa* of human clinical samples were collected from the microbiology laboratory unit among inpatients [250] and outpatients [250] attending Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AE-FUTHA) during a period of 12months. The isolates were further isolated on *Pseudomonas* Isolation Agar (PIA) (Merck Co., Germany) and incubated at 35◦C for 18–24 h. After incubation, *Pseudomonas aeruginosa* was identified by standard microbiology techniques such as colonial morphology, Gram staining, motility, and biochemical tests as previously described [1,14].

2.3 Screening for Multidrug-Resistant, Extensively Drug Resistant, and Pandrug-Resistant

This was done by disc diffusion technique on MHA according to the guidelines of the Clinical and Laboratory Standards Institute [15]. The following antibiotics were tested against the test bacteria: ceftriaxone (30 µg), Aztreonam (30 µg), cefotaxime (30 µg), tetracycline (30 µg), cefoxitin (30 µg), cefepime (10 µg), aztreonam (30 µg), amoxycillin clavulanic acid (20/10 µg), Piperacillin/tazobactam (30 µg), trimethoprim-sulphamethoxazole (125/23.75 µg), nalidixic acid (30 µg), colistin (10 µg) and amikacin (30 µg) (Oxoid, UK). Zones of inhibition diameters were measured, recorded, and interpreted as resistant or susceptible according to established guidelines [13,15]. Multidrug-resistant organisms (MDR) are described as acquired non-sensitivity to one or more agents in at least three groups of antimicrobials [14]. XDR = non-susceptible to ≥1 agent in all but ≤2 categories and Pan-Drug = non-susceptible to all antimicrobial agents listed [3,16].

3. RESULTS AND DISCUSION

3.1 Results

The result shows that the overall resistant phenotype was MDR 50. 7%, XDR 20.5%, and PDR 9.6% (Fig. 1) while isolates from in-patients and out-patients, resistant phenotype proportion was MDR 43.2%, XDR 32.4%, PDR 10.1% and MDR 61.2%, XDR 29.7%, PDR 18.5% respectively as presented in Fig. 2.

3.2 Discussion

Pseudomonas aeruginosa in both samples of in-patients and out-patients in multipurpose healthcare institutions had occurrence frequencies as high as 43.2–61.2%. Our finding agrees with reports from other studies [17-19]. Improper antibiotic prescriptions in our hospital could be a possible reason for these resistant features. Additionally, MDR-*P. aeruginosa* has been considered a serious threat by the Centers for Disease Control and Prevention (CDC) for the last decade, accounting for at least 32,600 cases, 2700 deaths, and US $767 million in attributable healthcare costs annually [1, 2, 20]. As stated in several studies, septicemia or pneumonia caused by MDR-*P. aeruginosa* isolates often result in poor patients outcome [21-25]. Recommendations for the treatment of MDR *P. aeruginosa* infections have been published based on the susceptibility profiles of conventional and novel antibiotics [17,26,27]. Clearly, antibiotic combination therapy is likely to select mutants displaying a broader resistance phenotype (e.g., mutational inactivation of the repressor gene mexR that regulates the multidrug efflux operon mexAB–oprM for *P. aeruginosa*) than before [28]. Furthermore, the proportion of patients who received delayed appropriate therapy increased as the number of resistant classes increased. Specifically, earlier report shows that, antibiotic resistance to at least three antibiotic classes was significantly associated with delayed appropriate therapy [29].

In this study, 29.7% and 32.4% XDR were found in the isolates from out-patients and in-patients. Similarly, the observed phenotype has been reported in Greece, Pakistan, Canada, and elsewhere [5,30-32]. In a US national database study, patients with *P. aeruginosa* respiratory infections had higher mortality, approximately seven days longer hospital length of stay (LOS), higher readmission rates, Multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Pseudomonas aeruginosa* represents a frequent

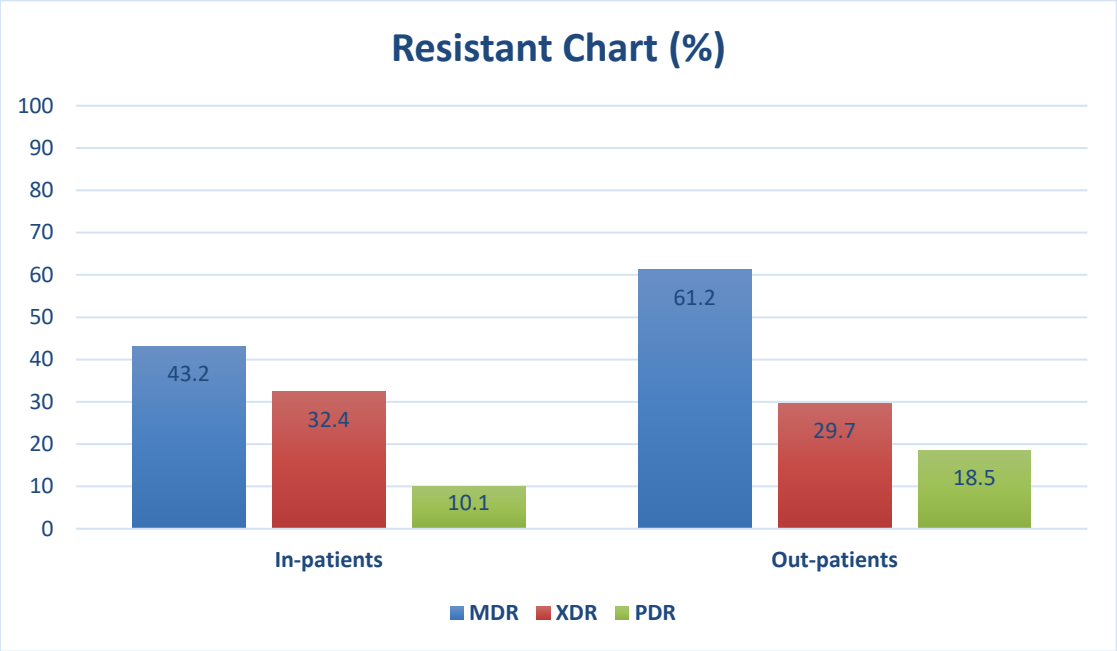

Fig. 1. Chart showing the proportion of MDR, XDR, and PDR among patient groups

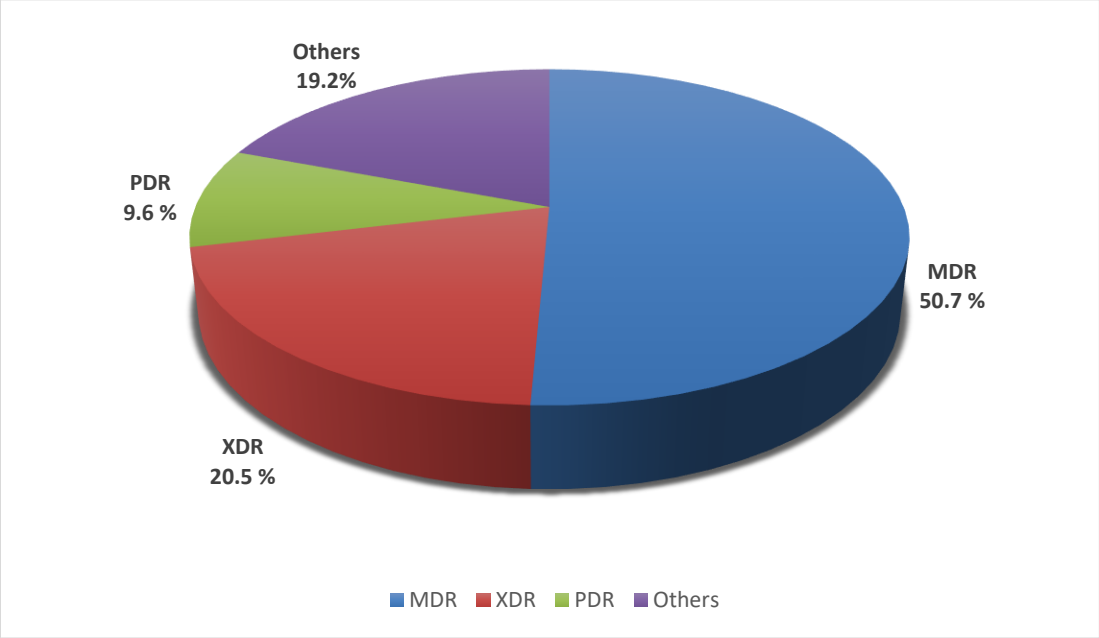

Fig. 2. Overall Patients Resistant type

and challenging nosocomial pathogen with consistently high rates that range from 11.5% to 24.7% and 9.0% to 11.2%, respectively, according to the INFORM database [33]. The World Health Organization (WHO) designated *P. aeruginosa* a priority 1 or ''critical'' pathogen in substantial need of new therapies to counteract this imminent public health crisis of resistance [29].

Resistant to all anti-pseudomonal drugs as pan-drug resistance had a proportion of 10.1% and 18.5% respectively. Infections caused by PDR *P. aeruginosa* strains could be associated with high morbidity and mortality rates, as well as increased durations of hospital stays and overall costs of treatment. PDR *Pseudomonas aeruginosa* has an extraordinary capacity to confer resistance via multiple mechanisms, often

at the same time, resulting in resistance to nearly all available antibiotics. Major PDR *P. aeruginosa* resistance mechanisms are often classified into intrinsic and acquired, which counter most antibiotics, as well as adaptive, which includes biofilm-mediated resistance and the formation of multidrug-tolerant persister cells [10,12,29,34]. In Nigeria, beta-lactams, cephalosporins, aminoglycosides, fluoroquinolones, tetracyclines, and folate pathway inhibitors are widely used in treating arrays of bacterial infections (such as UTIs, pneumonia, enteritis, and septicemia) and may likely result in selective pressure, thus favoring the evolution and development of MDR, XDR and PDR bacterial pathogens which suppresses the normal commensal bacteria.

P. aeruginosa high level of intrinsic resistance occurs through restricted outer membrane permeability (approximately 12–100-fold lower than that of *Escherichia coli*), the presence of antibiotic efflux systems, and the production of endogenous antibiotic-inactivating enzymes [29,34]. Acquired resistance mechanisms result from either horizontal gene transfer (acquisition of aminoglycoside-modifying enzymes and β-lactamases) or mutational events that result in the overexpression of efflux pumps or β-lactamases or the decreased expression or modification of target sites and porins [29,34]. Adaptive resistance mechanisms are induced by external stimuli (e.g., antibiotic exposure) and become inactive upon removal of the stimulus [29,34]. This resistant mechanism are the mainstay of progressive dissemination of MDR, XDR, and PDR among patients and may spread to another bacteria strain. It should be noted that, improper antimicrobial use, improper dose, and duration of administration as predisposing factors contributing to the emergence of MDR, XDR and PDR strains in a locality.

4. CONCLUSION

This present study reports the dissemination of MDR, XDR, and PDR among patients. Worrisomely, only few tested antimicrobial agent (Amikacin, cefepime) were susceptible, presenting a limited therapeutic option. The alarming high frequency of MDR, XDR, and PDR traits reported in this study poses significant concerns for treatment failures and emphasizes the urgent need for regulatory measures to curtail their dissemination and reduce their disease burden in healthcare settings. Proper medical guidance and avoidance of misuse of these antimicrobials agent in our study area should be adopted.

ETHICAL APPROVAL

Ethical approval with reference No: SMOH/ERC/043/22 was obtained from the Research and Ethics Committee of Ebonyi State Ministry of Health, Abakaliki, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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