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Prevalence of multidrug efflux pump requiring ciprofloxacin, ofloxacin and pefloxacin as substrates, among clinical isolates of *Pseudomonas aeruginosa*

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ABSTRACT

Forty two consecutive clinical isolates of *Pseudomonas aeruginos*a were screened for the presence of reserpine inhibited multidrug (MDR) efflux pump, utilizing ciprofloxacin ofloxacin and/or pefloxacin as substrates, by determining the minimum inhibitory concentration in the presence and absence of 100 mg/L reserpine. The result showed that 50% of the *Pseudomonas aeruginosa* isolates possessed reserpine inhibited MDR efflux pump. MDR efflux pump requiring ofloxacin (40.48%) were significantly ($p<0.01$) more among the isolates when compared with those requiring ciprofloxacin (16.67%) or pefloxacin (11.90%). Only one isolate possessed reserpine inhibited MDR efflux pumps that utilize all three fluiroquinolones. Research into suitable combination of antibacterials and appropriate pump mactivators or antibacterials that are less likely to be substrate for MDR pumps is advocated.

Keywords: Efflux pump, Reserpine, Fluoroquinolones, Pseudomones aeruginosa

INTRODUCTION

Efflux pumps are transport proteins involved in the extrusion of toxic substrates (including virtually all classes of clinically relevant antibiotics) from within cells into the external environment (Webber and Piddock, 2003). They are found in both procaryotes and eucaryotes (Bambeke *et al.,* 2003). Gram-negative bacteria are generally much more resistant than Gram-positive bacteria to a variety of antimicrobial agents (Li *et al.,* 2000). In the case of *Pseudomonas aeruginosa,* this has traditionally been attributed to the presence of a highly impermeable outer membrane (OM) (Nikaido, 1989). However, recent reports attribute synergy between low OM permeability and broadly specific drug efflux pumps to be responsible for *Pseudomonsa aeruginosa* intrinsic resistance (Li *et al.,* 2000; Zhang *et al.,* 2001; Chuanchuen *et al.,* 2002). Efflux pump can result in both intrinsic and acquired multidrug (MDR) resistance (Nikaido, 1998; Poole, 2000). Several MDR efflux systems have been described in *Pseudomonas aeruginosa,* including the MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexXY-OprM and MexJK-OprM (Li *et al.,* 2000; Chuanchuen *et al.,* 2002).

The susceptibility of *Pseudomonas aeruginosa* strains to antimicrobial agents can be significantly enhanced by pump inactivation (Li *et al.,* 1995) and reserpine – a plant alkaloid, has been used to inactivate MDR efflux pump (Neyfakh *et al.,* 1993; Schmctz *et al.,* 1998; Ribera *et al.,* 2002). The importance of detecting MDR efflux pump is to provide information for research into new drugs, coupling existing drugs to pump inactivators or modify existing drugs to make them less likely to be substrate for MDR efflux pump. A lot of report on MDR efflux pumps form various parts of the world exist, but there are none (to our knowledge) from Nigeria. Hence this study focuses on the prevalence reserpine inhibited MDR efflux pump of *Pseudomonas aeruginosa* to three most commonly prescribed fluoroquinolone in Nigeria.

MATERIALS AND METHODS

Bacterial Isolates

Forty two consecutive clinical isolates of *Pseudomonsa aeruginosa* obtained from the Medical Microbiology Department, University of Benin Teaching Hospital, Benin City, Nigeria, were used for this study. An isolate was idenfied as *Pseudomonsa aeruginosa* if it was a Gram-negative bacillus, oxidase positive, non-lactose fermenting motile, grows on 0.03% centrimide agar, production of pyocyarin on nutrient agar after 24 h incubation at 37 °C and growth at 42 °C.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) of ciprofloxacin (V.S. International PVT Ltd, India) ofloxacin (Nigeria German Chemicals, Nigeria) and pefloxacin (Rhone – Poulene, France) against the clinical isolates of *Pseudomonas aeruginosa* were determined using the two – fold serial broth (Muelier –Hinton Broth – Control S, Spain) dilution method with an inculum of 1 x 10^6 cells/mL. All experiments were repeated three times with

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and without 100mg/L reserpine (BDH Chemicals Ltd, England). The MIC was taken as the lowest concentration inhibiting visible growth after 18 h incubations at 37 °C.

Reserpine inhibited MDR effelux pump was inferred if the MIC with reserpine was four-fold or lower than the MIC without reserpine.

Chi (X^2) square test was used, manually, to determine significant difference.

Table 1: The effect of reserpine on the MIC some fluorquinolones

Strain number	*Pseudomonas aeruginosa MIC (mg/L)*					
	CIP	**CIP[a]**	**OFX**	**OFX [a]**	**PER**	**PEF [a]**
1	**320**	**80**	**640**	**160**	640	320
2	0.0391	0.0391	**20**	**0.0195**	0.0781	0.0781
3	40	20	160	80	640	640
4	1.25	1.25	2.5	2.5	0.625	0.3125
5	40	40	**160**	**40**	80	40
6	40	40	**320**	**80**	640	320
7	640	320	640	640	640	320
8	40	20	**320**	**80**	320	320
9	20	10	160	80	320	160
10	**10**	**0.0781**	10	10	40	40
11	640	320	**80**	**10**	**640**	**20**
12	5	2.5	**640**	**10**	20	20
13	320	320	640	640	640	640
14	0.1563	0.1563	0.625	0.625	0.625	0.625
15	320	160	640	640	640	640
16	**10**	**0.0391**	0.625	0.625	0.625	0.625
17	**10**	**0.0195**	10	10	**40**	**0.0195**
18	5	5	**80**	**10**	**640**	**10**
19	0.0195	0.0195	**320**	**10**	2.5	2.5
20	0.0391	0.0391	1.25	0.625	2.5	2.5
21	40	40	**320**	**0.0391**	640	640
22	40	40	320	320	640	640
23	320	160	640	320	640	640
24	40	40	640	320	320	320
25	80	40	80	80	320	320
26	640	640	640	640	640	640
27	80	80	160	80	320	320
28	80	40	320	160	320	320
29	**80**	**20**	**320**	**80**	320	320
30	640	320	**320**	**80**	640	320
31	320	320	**640**	**80**	640	640
32	**320**	**10**	320	160	160	80
33	**40**	**0.3125**	**80**	**20**	**320**	**40**
34	640	640	320	320	320	320
35	2.5	2.5	1.25	1.25	0.3125	0.3125
36	20	10	80	80	80	40
37	0.1563	0.1563	**10**	**0.1563**	0.1563	0.1563
38	0.1563	0.1563	0.1563	0.1563	0.1563	0.1563
39	0.1563	0.1563	0.1563	0.1563	0.1563	0.1563
40	0.625	0.3125	0.625	0.625	0.625	0.625
41	20	10	**20**	**0.0391**	**160**	**40**
42	320	160	**80**	**20**	320	320

Numbers in bold are MICs affected by reserpine by four – fold or more. CIP, Ciprofloxacin, OFX, Ofloxacin; PEF, Pefloxacin

[a]MIC of Fluoroquinolone in the presence of reserpine.

RESULTS AND DISCUSSION

The presence of reserpine inhibited MDR efflux pump was detected in 21(50%) of the 42 clinical isolates of *Pseudomonas aeruginosa* (Table 1). Reserpine has been shown not to inhibit all types of efflux pumps (Ribera *et al.,* 2002). Also, it has been reported that strains of *Staphylococcus aureus* very in the extent to which reserpine is able to block Nor A or other pumps (Schmitz *et al.,* 1998) and this is as a result of reserpine resistance, as has been observed in *Bacillus subtilis* (Ahmed *et al.,* 1993). These may partly explain the absence of reserpine MDR inhibited efflux pumps in some strains of *Pseudomonsa aeruginosa.* The other possible reasons include absence of reserpine inhibited pumps, and for the resistant strains, resistance may be due to other mechanisms such as decrease outer membrane (OM) permeability, mutation resulting in altered target site with less affinity for the drugs.

Only one isolate (2.38%) utilize all three fluorquinolones as substrate for efflux pump while 6(14.29%) utilize any two of the three fluoroquinolones (Table 1). Of the 6 isolates, 2 utilize ciprofloxacin and ofloxacin; 1 utilizes ciprofloxacin and pefloxacin while 3 utilize ofloxacin and pefloxacin as substrates. This further confirms multiple efflux pumps in *Pseudomonas aeruginosa* and indeed this organism possess several (Li *et al.,* 2000).

The prevalence of reserpine inhibited MDR efflux pump requiring ofloxacin as substrate (40.48%) was significantly ($P < 0.01$) higher than that requiring either ciprofloxacin (16.67%) or pefloxacin (11.90%) as substrate (Table 2). This indicates that resistance to ofloxacin, in comparison with the other two fluoroquinolones used, is most likely due to reserpine inhibited MDR efflux pump.

The MIC of ciprofloxacin in reserpine inhibited MDR efflux pump *Pseudomonsa aeruginosa* strains decrease by a range of 4 – 512 fold with 4 (57.14%) of the 7 strains exhibiting efflux pump becoming susceptible [using MIC breakpoint of ≤ 1 mg/L according to Andrews (2004)]; ofloxacin MIC decreased by a range of 4 – 8192 fold with 4 (23.53%) of the 17 strains exhibiting efflux pump becoming susceptible [using MIC breakpoint of ≤ 2mg/L according to Andrews (2004)] and for pefloxacin it decrease by a range of 4 – 2048 fold with 1 (20%) of the 5 strains becoming susceptible [no available breakpoint was found, but that for ofloxacin was used]. The result suggests that efflux pump activity in *Pseudomonsa aeruginosa* strains, functions at various levels. This has also been suggested in *Staphylococcus aureus* (Kaatz and Seo, 1995; Schmitz *et al.,* 1998).

Combination of any of the studied fluoroquinolomes with an MDR efflux pump inhibitor, such as reserpine, may be effective in the treatment of infections caused by resistant *Pseudomonosa aeruginosa* stains exhibiting MDR efflux pump. However, reserpine also affects eucaryotes and this makes it unsuitable (Bambeke, *et al.,* 2003). Thus, research should focus on new and safer inhibitors. Also, drugs that are less likely to be substrates for MDR efflux pumps should be developed.

Conclusively, 50% of the clinical isolates of *Pseudomonsa aeruginosa* used in this study, possess reserpine inhibited MDR efflux pump and ofloxacin was the drug most likely to be utilized as substrates.

Table 2: Prevalence of reserpine inhibited efflux pump

Fluroquinolones	Number tested	Number with inhibited efflux pump (%)
Ciprofloxacin	42	7 (16.67)
Ofloxacin	42	17 (40.48)
Pefloxacin	42	5 (11.90)

$X^2 = 9.4267$, $p < 0.01$

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