Detection of plasmid profile from MDR *Pseudomonas aeruginosa* isolate from wound infection.

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Cite this as: BMCJ 2 017;3(2):

Received June 6, 2016;
Accepted November 5, 2016

Abstract

Background: Antibiotic resistance is a major problem in treating infections in hospitals. Wound infections are one of the most common infections in hospitals and *Pseudomonas aeruginosa* is a predominant isolate which is usually multi drug resistant. **Objective:** To find out the antibiotic resistance pattern and plasmid profiling of resistant *Pseudomonas aeruginosa* isolated from wound infection. Methods: A descriptive type of cross sectional study was carried out in the Department of Microbiology of Rajshahi Medical College and the Department of Botany of Rajshahi University, Rajshahi during the period from July 2014 to June 2015. A total of 150 wound swabs were collected from patients admitted in surgery department and its allied branches and cultured on appropriate bacteriological culture media. **Results:** Culture had yielded growth in 131(87.33%) cases and *Pseudomonas aeruginosa* was 27(18%), *Staphylococcus aureus* was 22(14.66%), *Escherichia coli* was 56(37.33%), *Proteus spp.* was 19(12.67%), *Klebsiella spp.* was 7(4.67%) respectively. Antibiogram test was done on *Pseudomonas aeruginosa* with 7 different groups of antibiotics and found 4(14.81%) were resistant to 3 groups of antibiotics, 2(7.41%) were on 4 groups, 5(18.52%) were on 5 groups, 10(37.04%) were on 6 groups and 6(22.22%) were on 7 groups. A total of 27(18%) isolates were resistant to 3-7 groups of antibiotics. These isolates were further tested for plasmid detection and plasmid was responsible in 19(70.37%) resistant cases. Conclusion: All wound infections should be treated after performing antibiogram with adequate doses and duration.

Key words: Wound infection, *Pseudomonas aeruginosa,* Multidrug resistance, Plasmids, Hospital infection.

Introduction

Wound infections (WI) include skin and soft tissue infection. Intact skin protects the underlying tissue against colonization and invasion by bacteria. But loss of integrity of the skin provides a moist, warm and nutritive environment for bacterial colonization, proliferation leading to wound infection[1].Wound infections may be hospital acquired (nosocomial) or community acquired. Hospital acquired infections is about 5% to 34% in both developed and developing countries [2] and it may cause by both aerobic and anaerobic bacteria and fungus. Clinically WI may be traumatic, burn, surgical and bed sore (in diabetic). Whatever the clinical nature of infections, *Pseudomonas aerugenosa* is considered as a major hospital problem. This bacterium is frequently found in the hospital utility solutions, tap water, sink, mops, detergents, respiratory and physiotherapy equipments etc.[3] Hospital personnel may transmit this bacteria from patient to patient while handling patients. It also causes septicemia, urinary tract, respiratory tract and great variety of systemic infections Reported incidences of nosocomial pneumonia was 16%, urinary tract infection was 12%, wound infection was 17-26% and septicemia was 10% [4]. *Pseudomonas* is an opportunist pathogen with resistance to â-lactams, quinolones, chloramphenicol and tetracyclines. It also develops resistance due to the production of enzymes (cephalosporinase), active efflux, very low permeability and poor affinity for the target [5]. These resistant is due to mutation in chromosomal genes, acquisition of resistant genes from same or different species of bacteria via plasmids or transposons genes by conjugation and transduction [6]. All these mechanisms make *Pseudomonas* most difficult bacteria to treat. A large number of acquired resistance genes for â-lactamases, extended-spectrum â-lactamases and

metallo-â-lactamases have been detected in *Pseudomonas* [7]. In recent years, increase prevalence of multidrug resistance in *P. aeruginosa* has been noticed. A limited number of antibacterial agents such as ticarcillin, piperacillin, cephalosporins, carbapenems and fluoroquinolones are effective against *P. aeruginosa*. Aminoglycosides are also used as a part of combination therapy [8]. So the present study has been carried out to determine pathogens responsible for wound infection, antibacterial resistance pattern of *P. aeruginosa* and detection of plasmids for resistant.

Table 1: Antibacterial resistance of *Pseudomonas aeruginosa*. N=27

Antibacterial groups .	Isolated number of *Pseudomonas aeruginosa*																									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Cephalosporins: Ceftriaxone(30µg)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	S	R	R
Carbapenem: Meropenem(10µg)	S	S	S	S	S	S	S	S	R	S	S	S	S	R	R	R	R	R	R	R	S	S	S	S	R	S	S
Aminoglycoside: Gentamicin (10µg)	R	R	R	R	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R
Monobactam: Aztreonam (30µg)	R	R	R	R	S	S	R	S	S	R	R	R	S	R	S	R	R	R	R	R	R	S	R	R	S	S	R
Tetracycline: Tigecycline (15µg)	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R
Fluoroquinolone: Ciprofloxacin(5µg)	R	R	R	R	R	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R
Penicillin: Ticarcillin(75µg)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R
No. of resistant.	6	6	6	6	4	3	3	3	5	6	6	6	5	7	6	7	7	7	7	7	6	5	5	4	3	5	6

Note: R= Resistant; S= Sensitive

Materials and Methods

This study was conducted in the Department of Microbiology of Rajshahi Medical College, Rajshahi during the period from July 2014 to June 2015. A total of 150 wound swabs were collected from patients admitted in Surgery Department and its allied branches. The samples were cultured on blood agar, nutrient agar and MacConkey's agar. *Pseudomonas aeruginosa* was identified by its colony morphology, microscopy, motility, pigment production, fruity odour and oxidase positivity. Antimicrobial susceptibility test was performed on Mueller-Hinton agar media with 7 different groups of commercially available antibiotics by disc diffusion method. Tested antibiotic discs were meropenem (10µg), ciprofloxacin (5µg), ceftriaxone (30µg), aztreonam (30µg), gentamicin (10µg), tigecycline (15µg) and ticarcillin (75µg). The result was reported as sensitive or resistant according to CLSI, 2012 recommendation. The strains which showed resistance to more than 3 different groups of

Table 2: Multidrug resistance patterns of *Pseudomonas aeruginosa*. N= 27

Resistant *Pseudomonas aeruginosa.*	No. of antibacterial drugs in group.				
	3 N(%)	4 N(%)	5 N(%)	6 N(%)	7 N(%)
	4(14.81)	2(7.41)	5(18.52)	10(37.04)	6(22.22)

antibiotics were considered as multidrug resistant (MDR). The MDR strains were tested for plasmid detection.

Plasmid DNA Extraction and Gel electrophoresis

Plasmid extraction was carried out by using the alkaline lysis method. Plasmids were then electrophoresed on 1% agarose gel in a horizontal tank at a constant voltage of 100V for 60 minutes. After electrophoresis, plasmid DNA bands were viewed under UV transillumination and photographed using a digital camera. The DNA bands were compared with those for the lambda DNA *Hind*III digest molecular weight marker (Promega Corporation) which ranged in size from 250bp to 10000bp and results recorded.

Results

In this study 27(18%) *Pseudomonas aeruginosa* was isolated from 150 wound swabs. Among 27 isolates, ceftriaxone was resistant to 25(92.59%), meropenem was 9(33.33%), gentamicin was 22(81.48%), aztreonam was 18(66.67%), tigecycline was 25(92.59%), ciprofloxacine was 22(81.48%) and ticarcillin was resistant to 26(96.3%) isolates (Table-I). Among 27 *Pseudomonas aeruginosa*, 4(14.81%) were resistant to 3 groups of drug, 2(7.41%) were 4 groups, 5(18.52%) were 5 groups, 10(37.04%) were 6 groups and 6(22.22%) were resistant to 7 groups of drug (Table-II). The plasmid was detected in 19(70.37%) isolates of which one in 3 groups , another one in 4 groups, four in 5 groups, seven in 6 groups and six in 7 groups of drugs (Table-III). swabs were collected from patients admitted in Surgery Department and its allied branches. The samples were cultured on blood agar, nutrient agar and MacConkey's agar. *Pseudomonas aeruginosa* was identified by its colony morphology, microscopy, motility, pigment production, fruity odour and oxidase positivity. Antimicrobial susceptibility test was performed on Mueller-Hinton agar media with 7 different groups of commercially available antibiotics by disc diffusion method. Tested antibiotic discs were meropenem (10µg), ciprofloxacin (5µg),

Table 3 : Correlation of multidrug resistant strains of *Pseudomonas aeruginosa* with detected plasmid. N=27

No. of antibiotic group N	No. of MDR isolates N (%)	Proportion of MDR isolates which carried plasmid % (N/n)
3	4(14.8)	25.0 (1/4)
4	2(7.4)	50.0 (1/2)
5	5(18.5)	80.0 (4/5)
6	10(37.0)	70.0 (7/10)
7	6(22.2)	100.0 (6/6)
Total	27(100.0)	70.4 (19/27)

Discussion

In this study seven groups of antibiotics were studied for sensitivity test. The groups were cephalosporin, carbapenem, aminoglycoside, monobactam, tetracycline, fluoroquinolone and penicillin. Among cephalosporins, ceftriaxone was 92.59% resistant which is similar with the study of Rostamzadeh *et al.* (2016) in Iran[9] and Mahmoud *et al.* (2013) in Egypt[10] where ceftriaxone resistant was 94.37% and 87.7%. But dissimilarity was found with the study of Garba *et al.* (2012)[11] and Mohammed *et al.*

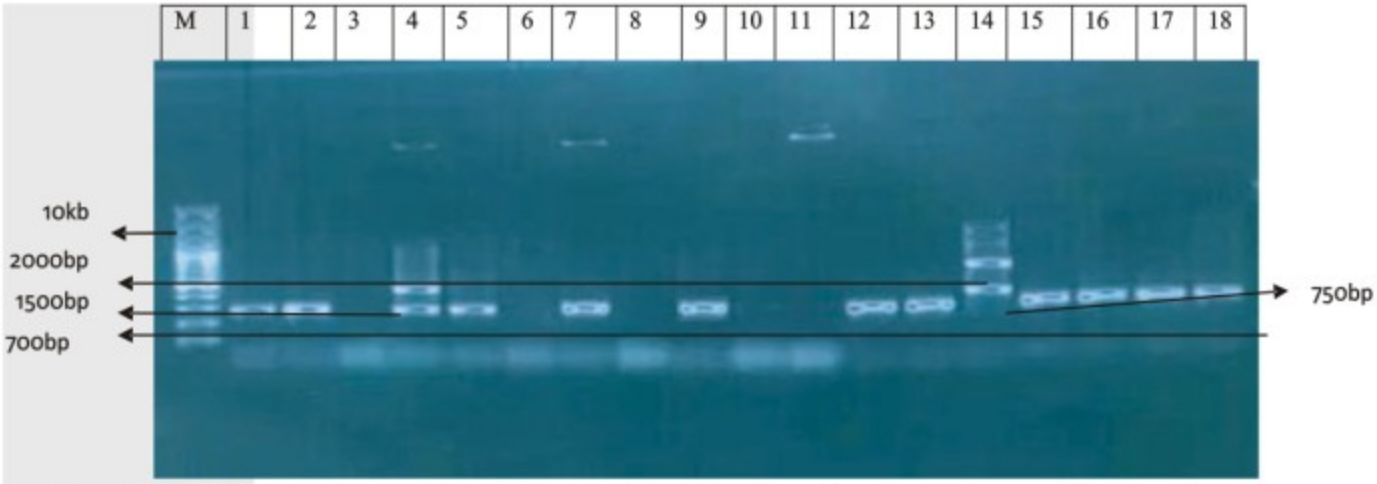

Plate 1: Plasmid profile of the MDR *Pseudomonas aeruginosa* isolates: 1,2,5,7,9,12,13,15,16,17,18(700bp), 4(700bp,1500bp),14(750bp,2000bp) is the clinical isolates. Lane M, 10kb DNA ladder.

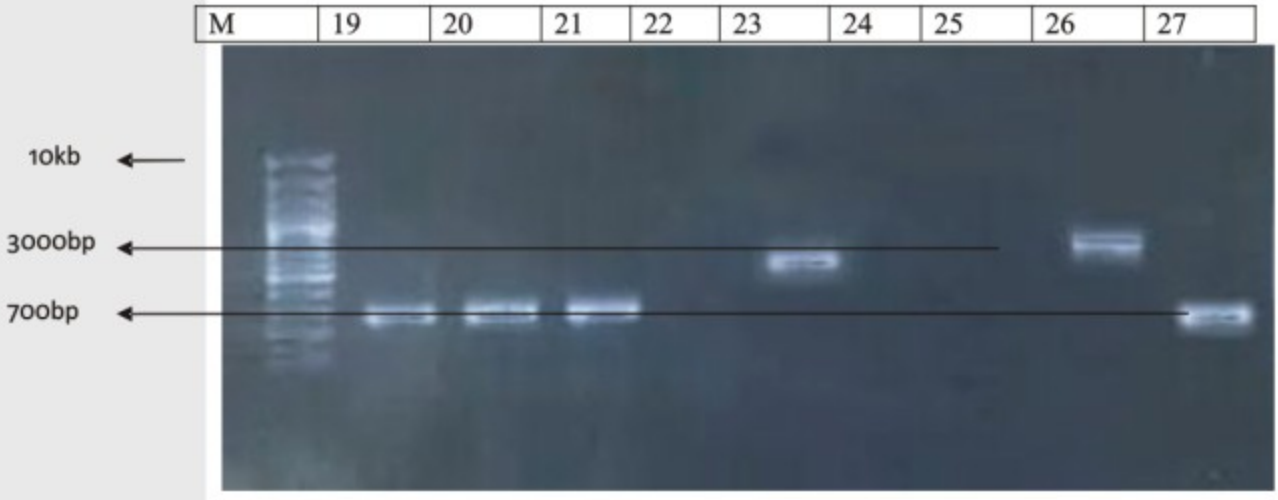

Plate 2: Plasmid profile of the MDR *Pseudomonas aeruginosa* isolates: 19,20,21,27(700bp), 23 and 26(3000bp) is the clinical isolates. Lane M, 10kb DNA ladder.

(2013)[12] both were in Nigeria and showed ceftriaxone resistant was 45.4% and 46%. Among the carbapenems, meropenem was 33.33% resistant which is similar with the study of Joseph *et al.* (2013) in India[13] and Khan *et al.* (2014) in Pakistan[14] where meropenem resistant was 34.8% and 30.4%. The dissimilarity noted by Yasemin *et al.* (2013) in Turkey[15] and Biswal *et al.* (2014) in India[16] where rates were 19% and 13.79%. Gentamicin was 81.48% resistant which is similar to the study of Rajput *et al.* (2015)[17] and Biswal *et al.* (2014)[16] both were in India where gentamicin resistant was 81% and 81.03%. Dissimilarity with our study was reported by Shah *et al.* (2015) in Pakistan[18] and Yasemin *et al.* (2013) in Turkey[15] where resistant was 35.3% and 36%. Aztreonam was 66.67% resistant which is nearly similar to the study of Nazli *et al.* (2015) in Turkey[19] where aztreonam resistant was 56.7% and dissimilar with the study of Khan *et al.* (2014) in Nepal[20] and Mahmoud *et al.* (2013) in Egypt[10] where resistant was 31.96% and 82.5%. Tetracycline was 92.59% resistant which is similar with the study of Smith *et al.* (2012) in Nigeria[21] and Mohiuddin *et al.*

(2010) in Dhaka, Bangladesh[22] where resistant was 95% and 91.17%. But our study is dissimilar with the study of Akingbade *et al.* (2012) in Nigeria[23] and Masood & Zahra (2014) in Iran[24] where resistant rates were 70.9% and 72%. Ciprofloxacin was 81.48% resistant which is nearly similar to the study of Mohiuddin *et al.* (2010) in Dhaka, Bangladesh[22] and Khan *et al.* (2014) in Pakistan[14] where ciprofloxacin resistant were 92% and 75%. But dissimilarity with our study was reported by Mahmoud *et al.* (2013) in Egypt[10] and Golshani *et al.* (2012) in Iran[25] were 56.1% & 58%. Ticarcillin was 96.3% resistant which is similar to the study of Shahini *et al.* (2012)[26] and Ranjbar *et al.* (2011)[27] both in Iran where resistant rates were 100% and 93%. But dissimilarity was reported by Sarwat *et al.* (2015) in India[28] & Masood and Zahra (2014) in Iran[24] was 58.46% and 5%.

The resistant pattern of *Pseudomonas aeruginosa* in our study is different with the studies of others may be due to the random use of 3rd generation cephalosporins and carbapenem without doing culture and sensitivity which lead to the emergence of resistance and their dissemination throughout the hospital. This dissemination is due to inadequate sanitation of hospital, improper use of antibiotics, inadequate antibiogram of empirical antibiotics, inadequate dose and duration, may be insufficient ingredients as mention by the pharmaceutical company and inaccuracy of culture and sensitivity test.

In our study 14.81% *Pseudomonas aeruginosa* was resistant to 3 antibiotics which is similar to the study of Gobedo *et al.* (2013) in Ethiopia[29] where they found 14.9%. Dissimilarity with our study was reported by Biswal *et al.* (2014)[16] in India where resistant isolates were 10.34%.

7.41% isolates were resistant to 4 antibiotics which are similar to the study of Yakha *et al.* (2014) in Nepal[30] and Odumosu *et al.* (2013) in Nigeria[31] where resistant isolates of both were 6.45%. Dissimilarity was reported by Biswal *et al.* (2014) in India[16] were 3.45%. In this study 18.52% isolates were resistant to 5 antibiotics which are similar to the study of Mehdi *et al.* (2014) in Iran[32] and Yakha *et al.* (2014) in Nepal[30] where resistant isolates were 17.8% and 19.35%. Dissimilarity was reported by Gobedo *et al.* (2013) in Ethiopia[29] were 4.1%. In this study 37.04% isolates were resistant to 6 antibiotics which are similar to the study of Mehdi *et al.* (2014) in Iran[32] were 38.4%. But dissimilarity was reported by Odumosu *et al.* (2013) in Nigeria[31] were 9.68%. In our study 22.22% isolates were resistant to 7 antibiotics which are dissimilar with the study of Gobedo *et al.* (2013) in Ethiopia[29] where resistant isolates were 5.4%.

The dissimilarities of the multidrug (3-7 drugs) resistant isolates may be due to use of antibiotics in our study is different from others study, different therapeutic dose and route; patients may have different pH in their stomach which may differ the activity of orally administered drugs like ciprofloxacin; food can interfere the absorption of drug e.g. tetracycline; milk, antacid, sucralfate and iron salt may reduce the absorption of certain drugs e.g. tetracycline, fluoroquinolone etc. Dissimilarities may also be due to achlorhydia, partial gastrectomy, tropical sprue where absorption of drug reduce and cannot reach at optimum serum concentration. In oral administration as only 20-40% drug reaches the systemic circulation while 100% in parenteral administration, metabolism may also alter the efficacy and half-life of drug. Besides these oral formulation of a drug from different manufacturers or different batches from the same manufacturer with same amount of drug may not yield the same blood levels. Mutation may occur in bacteria if optimum blood level is not attained by orally administered drug that also causes antibiotic resistance.

In this study, plasmid was detected in 19 (70.37%) isolates out of 27 MDR strains which is nearly similar with the study of Daini *et al.* (2008)[33], Smith *et al.*

(2012)[21] and Daini & Onyeaghala (2012)[34] all were from Nigeria where detected plasmid were 66.67%, 80% and 81.48%. Dissimilarity with our study was also reported by Akingbade *et al.* (2012) in Nigeria[23] and Afrin. (2015) in Bangladesh[35] where detected plasmid was 36.4%, and 50%.

The dissimilarities of plasmid detection may be due to inter species dispersion of plasmid, the presence of transmissible and non-transmissible plasmids. It has been seen in last two decades that bacterial resistance to a large number of antibiotics may be transfer by plasmids (Hasan *et al.* 2007)[36]. In our study, no plasmid had been detected in 8(29.63%) isolates and it is nearly similar with the finding reported by Quashem (1987) in Bangladesh[37] who reported plasmid in 30.59% isolates. There is a possibility that some of the plasmids were lost due to 6 months storage of the MDR samples at 20^0C before test. Loss of plasmid due to storage has been reported also by many workers (Watanabe *et al.*, 1964)[38]. The failure of plasmid detection were also might be the cause of that the resistance determinants were either carried by chromosomes or by small molecular weight plasmids.

It can be concluded that though a proportion of multidrug resistance can occur due to mutation in chromosomal DNA but plasmid bearer always play an important role in antibiotic resistance. From this study, it has found that *Pseudomonas aeruginosa* isolates are resistant to commonly used antibiotics and its resistance to antimicrobials are gradually increases day by day. Therefore the rational use of antibiotics must be a priority. Public health policy on appropriate prescribing and antibiotics should be used only after performing antibiogram with adequate dose and duration.

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