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PHENOTYPIC AND GENOTYPIC DIVERSITY OF *PSEUDOMONAS AERUGINOSA* STRAINS ISOLATED FROM HOSPITALS IN SIEDLCE (POLAND)

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ABSTRACT

A total of 62 *Pseudomonas aeruginosa* strains isolated from two hospitals in Siedlce (Poland) were studied by repetitive element based PCR (rep-PCR) using BOX primer. BOX-PCR results revealed the presence of 7 numerous genotypes and 31 unique patterns among isolates. Generally, the strains of *P. aeruginosa* were characterized by resistance to many antibiotics tested and by differences in serogroups and types of growth on cetrimide agar medium. However, the *P. aeruginosa* strains isolated from faeces showed much lower phenotypic and genotypic variations in comparison with strains obtained from other clinical specimens. It was observed that genetic techniques supported by phenotypic tests have enabled to conduct a detailed characterization of *P. aeruginosa* strains isolated from a particular environment at a particular time.

Key words: *Pseudomonas aeruginosa*, BOX-PCR, antibiotic resistance, serotyping, cetrimide agar.

INTRODUCTION

Pseudomonas aeruginosa is a ubiquitous pathogen prevalent in hospital environments. It can cause severe nosocomial infections, particularly among immunocompromised patients. People with respiratory, gastrointestinal, urinary tract, and wound infections as well as burn victims, individuals with cancer, and patients hospitalized in intensive care units are affected by *P. aeruginosa* mostly due to nosocomial spread and cross contaminations (9, 10, 14). *P. aeruginosa* accounts for 10% of all hospital acquired infections, a site specific prevalence which may vary from one unit to another and from study to study (11). Various possible sources of *P. aeruginosa* infection in hospitals have been identified, *i.e.*, tap water, disinfectants, food, sinks, mops, medical equipment, hospital personnel and others (7, 14, 19).

P. aeruginosa can be internally divided into subgroups by classical methods such as: biotyping, serotyping, pyocin typing, phage typing and antibiotic sensitivity of tested strains. However, the discriminatory power is much lower than that obtained by molecular typing methods. DNA typing methods have been frequently used to investigate the diversity of collections of *P. aeruginosa* (20). These methods include pulsed-field gel electrophoresis (PFGE) (8, 21, 22), ribotyping (6, 8), restriction fragment length polymorphic DNA analysis (RFLP) (6), random amplified polymorphic DNA assay (RAPD) (8, 13, 21), arbitrary primed PCR (AP-PCR) (4), amplified fragment length polymorphism (AFLP) (21), and repetitive element based PCR (rep-PCR) (6, 22). Rep-PCR is a method for fingerprinting bacterial genomes, which

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examines strain-specific patterns obtained from PCR amplification of repetitive DNA elements present within bacterial genomes. Three main sets of repetitive elements are used for typing purposes: the repetitive extragenic palindromic (REP) sequence, the enterobacterial repetitive intergenic consensus sequence (ERIC) and the BOX elements (16).

The aim of this work was to estimate intra-species differentiation of *P. aeruginosa* strains isolated from two hospitals in Siedlce (Poland) using phenotypic methods (serotyping, susceptibility to chemotherapeutic agents, and type of growth on cetrimide agar medium) and the genotypic method (BOX-PCR).

MATERIALS AND METHODS

Bacterial strains

A total of 62 strains of *P. aeruginosa*, were originally isolated from a variety of clinical specimens: faeces (26), urine (12), blood (1), bronchial washings (8), sputum (1), wound swab (9), throat swab (2), ulceration swab (1), swab from skin round tracheotomy (1) and from ear (1). The bacteria were obtained from 62 patients from different wards of the municipal hospital, main hospital and outpatients´ department in Siedlce (Poland), between December 2005 and March 2006. The strains were identified as *P. aeruginosa* on the basis of typical morphology by gram-negative staining, a positive oxidase reaction, growth at 42°C and conventional biochemical tests using the Api 20NE system (Bio-Mérieux, France). We also identified *P. aeruginosa* by PCR amplification of 16 S ribosomal RNA (12). All isolates resulted in a positive reaction. The control strain of *P. aeruginosa* NCTC 6749 was also examined. Stock cultures were stored in tripticase soy broth (TSB, Difco, USA) containing 20% glycerol at -80°C.

Genetic analysis

Isolates were grown in TSB at 37°C for 24 h and DNA was extracted by using the Genomic DNA Pre Plus (A&A Biotechnology, Poland). Rep-PCR fingerprinting was carried out using one BOX primer of sequence 5' - CTA CGG CAA GGC GAC GCT GAC G - 3'. Amplification was carried out with a 10x PCR buffer (100 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 100 mM KCl, 0.5% Nonidet P40, 0.5% Tween 20) in a total reaction of 50 µL containing 2.5 mM dNTP, 20 mM $MgCl_2$, 100 pmol of primer, 2 µL of genomic template DNA, and 1 unit of Taq DNA polymerase (DNA Gdansk, Poland). Rep-PCR typing was carried out according to Dawson *et al.* (6) using a PTC-100 Programmable Thermo Controller (MJ Research, USA) according to the following procedure. Initial denaturation at 94°C for 5 min followed by 35 cycles of PCR consisting of denaturation at 94°C for 1 min, annealing at 48°C for 2 min, and extension at 72°C for 2 min; in the last cycle, the extension time was 5 min. The PCR product (10 µl) was analysed using a 2% agarose gel in the TBE buffer [5.4 g l^{-1} Tris, 2.75 g l^{-1} Boric acid, 0.37 g l^{-1} EDTA (pH 8.0)] and photographed under the UV light. The size of the products was analyzed in comparison to a M100-1000 bp ladder M.W. size marker (DNA Gdansk, Poland).

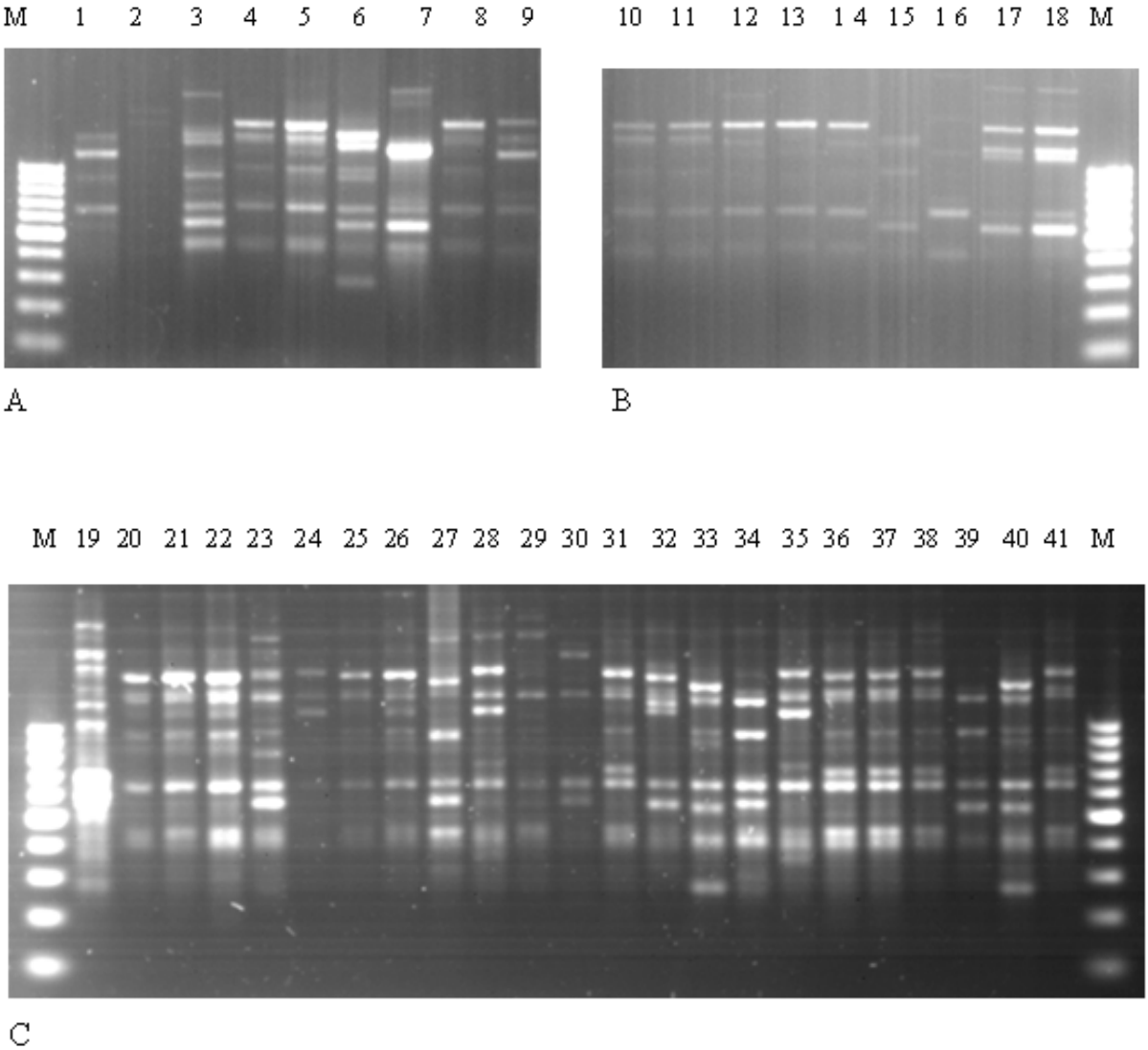
Phenotypic study

Pyocin production was tested on selective Cetrimide Agar (Merc, Germany). Serotyping was determined by the slide agglutination test with 16 monovalent antisera numbered O1 to O16 and 4 polyvalent antisera [PMA (O1 + O3 + O4 + O6), PME (O2 + O5 + O15 + O16), PMF (O7 + O8 + O11 + O12), PMC (O9 + O10 + O13 + O14)] (Sanofi Diagnostics Pasteur, France) as recommended by the manufacturer. Susceptibility to antibacterial drugs was studied by the disk diffusion method according to CLSI (Clinical and Laboratory Standards Institute) (3) for 12 following antimicrobial agents (Bio-Mérieux, France): carbenicillin (CB, 100 µg), mezlocillin (MZ, 75 µg), piperacillin (PIP, 100 µg), piperacillin-tazobactam (TZP, 100 µg+10 µg), aztreonam (ATM, 30 µg), ceftazidime (CAZ, 30 µg), imipenem (IMP, 10 µg), meropenem (MEM, 30 µg), gentamicin (CN, 10 µg), netilmicin (NET, 30 µg), amikacin (AN, 30 µg) and ciprofloxacin (CIP, 5 µg).

RESULTS

BOX-PCR fingerprinting revealed 38 genetic patterns, among them 7 main genotypes, containing 3 to 8 isolates and 31 other unique patterns. The clusters were shown in 2 to 11 bands between 280-1550 bp. Over half of the isolates had 5 to

8 bands per pattern. The most characteristic products of PCR for *P. aeruginosa* were the following: 200, 420, 650, 1200 and 1400 bp (Fig. 1). Two of the genotypes (8 and 21) consisted of 7 (11.3%) and 8 (12.9%) isolates, respectively. The next two numerous genotypes (4 and 13) contained 4 (6.45%) isolates. All these isolates were obtained from faeces of patients hospitalized in the Paediatric Ward of the Main and Infectious Ward of the Municipal hospitals. The remaining three numerous genotypes (11, 23 and 5) consisted of isolates from wound (3 isolates) of patients of Orthopaedic and Orthopaedic-Traumatical Ward; bronchial washings (3) of patients of Neurological Ward, and from urine (2), and wound (1) of patients being treated in Orthopaedic, Urologic and Rehabilitation wards of the Main Hospital. Other unique types were collected from the following clinical specimens: urine (83.3%), wound (55.5%), bronchial washings (62.5%), faeces (11.5%) and from sputum (1), throat swab (2), ulceration swab (1), swab from skin round tracheotomy (1), blood (1), and from ear (1). This data demonstrated that isolates from urine, wound and bronchial washings were highly heterogeneous; among 12, 9 and 8 isolates, 11, 7 and 6 respectively different clusters appeared. While the group of isolates from faeces showed slightly genetic variation; in the group of 26 isolates we detected 7 genotypes.



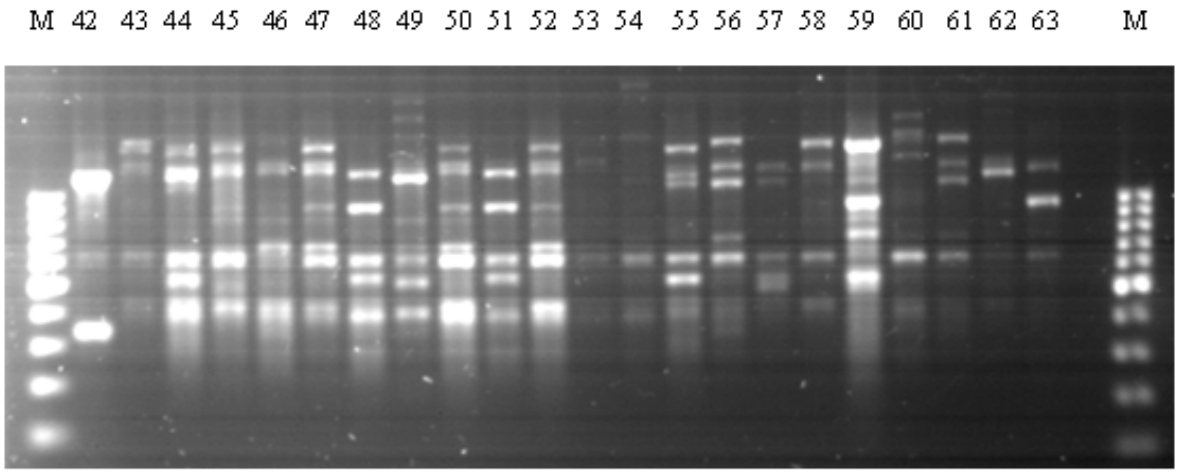


Figure 1. BOX-PCR fingerprinting of *Pseudomonas aeruginosa* strains. Lane M: Molecular weight marker (MW100-1000 bp, DNA-GDANSK). A - Lines 1 to 9 - *P. aeruginosa* strains isolated from urine (1, 2, 6, 7), wound (3) and faeces (4, 5, 8). B – Lines 10 to 18 - *P. aeruginosa* strains isolated from faeces (10, 11, 12, 13, 14), NCTC 6749 (15) and wound (16, 17, 18). C – Lines 19 to 41 - *P. aeruginosa* strains isolated from bronchial washings (19, 28, 34, 35), faeces (20, 21, 22, 25, 26, 31, 36, 37, 38, 41), throat swab (23), skin (24), wound (27, 29) and urine (30, 32, 39). D – Lines 42 to 63 - *P. aeruginosa* strains isolated from throat swab (42), faeces (43, 45, 47, 50, 52, 53, 59), urine (44, 49, 57, 62), wound (46, 55), bronchial washings (48, 51, 56, 61), ulceration (54), blood (60) and ear (63).

Detailed data on comparison of genotypic and phenotypic strain features are presented in Table 1.

All tested strains were agglutinable. Forty three (69.3%) of 62 strains gave agglutination with the monovalent O6 serum. They were isolated from faeces (100%), urine (66.7%), bronchial washings (37.5%), wound (44.4%) and single strains from throat swab and swab from skin round tracheotomy. Six (9.7%) strains obtained from wound (33.3%), urine (16.7%) and sputum (1) reacted with serum O1. Eight (12.9%) strains isolated from bronchial washings (50.0%), wound (22.2%), throat swab (1) and ulceration (1) were typed only by polyvalent sera: PMA (5), PMF (2) and PMC (1). Individual isolates from urine, bronchial washings and blood were assigned to following sera: O9, O10, O15 and O16. A variety of serotypes were demonstrated among 12 isolates from urine (O6, O1, O9, O10, PMA), 9 isolates from wound (O6, O1, PMF) and 8 isolates from bronchial washings (O6, O15, PMA). While 26 of the strains isolated from faeces were typed only by one sera (O6). Four different serotypes (O6, O1, O15, PMC) were observed among 9 isolates from patients hospitalized in the Intensive Care Unit Ward of the Main Hospital, whereas all strains isolated from patients of the Infectious Ward (17 isolates) of the Municipal Hospital and the Paediatric Ward (10 isolates) of the Main Hospital belonged to one (O6) serotype. There was correlation between serotypes and genotypes of *P. aeruginosa* strains. The strains belonging to the same serotype were classified to the same genotypic type (PMA serotype – genotype 23; O1 serotype – genotype 5), however O6 serotype was classified to four genotypes: 4, 8, 13 and 21 .

The total of 62 *P. aeruginosa* strains were tested on selective cetrimide agar. A celadon type of growth appeared most frequently; 38 (61.3%) strains. These strains were isolated mainly from faeces, urine and wound (92.3%, 58.3% and 44.4% respectively). Eleven (17.7%) strains produced green colonies. Most of them were isolated from bronchial washings (50.0%) and wound (33.3%). Seven (11.3%) strains isolated from bronchial washings (37.5%), urine (25.0%) and wound (11.1%) grew in cetrimide agar producing green-yellow colonies. Blue and green-blue types of growth were most rarely found, 6.45% and 3.2% respectively. *P. aeruginosa* strains

isolated from the faeces of patients being treated at the Infectious Ward of the Municipal Hospital, and the Paediatric Ward of the Main Hospital produced nearly 90% and 100% celadon colonies respectively. While the strains isolated from the other clinical specimens of patients hospitalized in different wards (excluding the Paediatric ward) of the Main Hospital produced this type of growth by a much lower degree (47.5%). Six out of seven numerous genotypes consisted of strains that grew on selective cetrimide medium producing celadon type (with exception of two strains). Only the strains isolated from bronchial washings of genotype 23 produced green colonies.

The majority of *P. aeruginosa* isolates showed much differentiated resistance to antimicrobial agents tested. Different resistance patterns in various arrangements were observed from sensitivity to all tested antibiotics, through resistance to only two or three antibiotics, to multidrug resistance for almost all tested drugs. Strains isolated from faeces (serotype O6) of patients hospitalized in the Infectious Ward of the Municipal Hospital and the Paediatric Ward of the Main Hospital, were generally less resistant to chemotherapeutic agents than strains isolated from the other clinical specimens obtained from patients being treated in different wards (excluding the Paediatric ward) of the Main Hospital (CB-53.8%/72.2%, MZ-88.5%/86.1%, PIP-3.8%/30.55%, TZP-0%/19.4%, ATM-57.7%/19.4%, CAZ-15.4%/19.4%, IMP-3.8%/25%, MEM-7.6%/38.9%, CN-46.1%/72.2%, NET-42.3%/86.1%, AN-26.9%/38.4% and CIP-0%/25%). Among studied strains, 14 (22.3%) were multidrug resistant (MDR). They were resistant to at least 4 out of the 6 antipseudomonal classes of antimicrobial agents, *i.e.*, antipseudomonal penicilins, monobactams, cephalosporins, carbapenems, quinolones and aminoglycosides. These strains were obtained from wound (33.3%), urine (25.0%), bronchial washings (25.0%), faeces (11.5%) and individual isolates from sputum, blood and ear of patients hospitalized in different wards of the Main Hospital (11 strains) and the Municipal Hospital (1) as well as the outpatients´ department (2). They belonged to the following serotypes: O1, O6, PMA, PMF, O15, O16. However most of them were serotype O1 (35.7%). Results of antibiotic resistance and genotyping showed poor correlation. Resistance patterns from bacterial isolates which had identical genotypes differed in up to 9 antibiotics.

Table 1. Source of the isolation, hospital/ward, genotypes of clinical *P. aeruginosa* isolates and their phenotypic differentiation.

Genotype	No. of isolates	Source of isolation	Hospital/ward*	Serotype	Characteristic of the colony	Resistance pattern
1	1	urine	A/1	O6	green-yellow	CB,MZ,CN,NET
2	2	urine	A/2	O6	green-yellow	CB,ATM,CAZ
3	3	wound	A/3	O1	celadon	CB,MZ,PIP,TZP,MEM, ATM,CAZ,CN,AN,ET
4	4	faeces	B/4	O6	celadon	CB,MZ,ATM,CN,NET
4	5	faeces	B/4	O6	celadon	CB,MZ,CN
4	8	faeces	B/4	O6	celadon	CB,MZ,ATM,CN,NET
4	12	faeces	A/8	O6	celadon	MZ,AN
5	6	urine	A/5	O1	celadon	CB,MZ,PIP,TZP,IMP, MEM,CN,AN,NET,CIP
5	33	urine	A/6	O1	celadon	CB,MZ,PIP,TZP,MEM, ATM,CAZ,CN,AN,NET,CIP
5	40	wound	A/3	O1	celadon	CB,MZ,PIP,TZP,MEM, ATM,CAZ,CN,AN,NET,CIP
6	7	urine	A/6	O10	green-yellow	MZ,MEM
7	9	sputum	A/7	O1	celadon	CB,MZ,IMP,MEM,ATM, CAZ,NET
8	10	faeces	A/8	O6	celadon	CB,MZ,CAZ,NET
8	11	faeces	B/4	O6	celadon	MZ,AN
8	13	faeces	B/4	O6	celadon	MZ,ATM,CN
8	14	faeces	B/4	O6	celadon	MZ,ATM,AN,NET

8	43	faeces	A/8	O6	celadon	CB,MZ,CAZ,AN,NET
8	45	faeces	B/4	O6	blue	MZ,CN
8	58	faeces	A/8	O6	celadon	CB,MZ,ATM,CN,NET
9	15	NCTC6749	-	O6	green-blue	CB,ATM,CAZ
10	16	wound	A/9	PMF	celadon	CB,MZ,PIP,TZP,IMP, MEM,CN,NET
11	17	wound	A/3	O6	geen	CB,MZ,PIP,TZP,CN, NET,CIP
11	18	wound	A/2	O6	green-yellow	CB,MZ,CN,AN,NET
11	55	wound	A/2	O1	green	CB,MZ,CN,AN,NET
12	19	bronchial washings	A/7	O15	blue	CB,MZ,PIP,TZP,IMP, MEM,ATM,CAZ,CN, AN,NET,CIP
13	20	faeces	B/4	O6	celadon	CB,PIP,ATM,CAZ,CN
13	21	faeces	B/4	O6	green	MZ,ATM
13	22	faeces	B/4	O6	celadon	CB,MZ,NET
13	25	faeces	A/8	O6	celadon	CB,MZ,CAZ
14	23	throat swab	A/7	O6	celadon	CB,MZ,NET
15	24	skin	A/7	O6	celadon	CB,MZ,IMP,MEM,NET
16	26	faeces	A/8	O6	green	MZ,MEM,ATM,CN,AN, NET
17	27	wound	A/2	PMF	celadon	CB,MZ,CN,NET
18	28	bronchial washings	A/7	O6	celadon	CB,MZ,CN,NET
19	29	wound	A/9	O6	green-yellow	MZ,CN,AN,NET
20	30	urine	C	O6	green-blue	CB,MZ,MEM,ATM, CAZ,CN,NET
21	31	faeces	B/4	O6	celadon	CB,MZ,IMP
21	36	faeces	B/4	O6	celadon	MZ,ATM,CN,NET
21	37	faeces	A/8	O6	celadon	MZ,ATM,AN
21	38	faeces	B/4	O6	celadon	sensitive to all
21	41	faeces	B/4	O6	celadon	CB,MZ
21	47	faeces	B/4	O6	celadon	MZ,ATM,CN
21	50	faeces	B/4	O6	celadon	CB,MZ,ATM,CAZ
21	52	faeces	B/4	O6	celadon	CB,MZ,ATM,CN
22	32	urine	C	O6	celadon	CB,MZ,NET
23	34	bronchial washings	A/10	PMA	green	CB,MZ,PIP,CN,NET,CIP
23	48	bronchial washings	A/10	PMA	green	CB,MZ,IMP,MEM,CN, AN,NET
23	51	bronchial washings	A/10	PMA	green	CB,MZ,CN,AN,NET,CIP
24	35	bronchial washings	A/7	O6	green	MZ,IMP,MEM,CN,NET
25	39	urine	A/10	O6	green	CB,MZ,CN,AN,NET
26	42	throat swab	A/7	PMC	green	CN,AN,NET,CIP
27	44	urine	C	O6	celadon	CB,MZ,MEM,CN
28	46	wound	A/9	O6	green	MZ,CN,AN,NET
29	49	urine	A/11	O9	green-blue	MZ,PIP,NET
30	53	faeces	A/8	O6	celadon	CB,MZ,MEM,ATM,NET
31	54	ulceration	A/9	PMA	blue	CB,MZ,NET,CIP
32	56	bronchial washings	A/7	O6	green-yellow	CB, MZ, IMP, CN,NET
33	57	urine	A/8	O6	celadon	CN, AN,NET
34	59	faeces	A/8	O6	celadon	ATM,CN,AN,NET
35	60	blood	A/12	O16	celadon	CB,MZ,PIP,IMP,MEM, NET
36	61	bronchial washings	A/7	PMA	green-yellow	CB,MZ,CN
37	62	urine	C	O6	celadon	CB,MZ
38	63	ear	C	PMA	blue	CB,MZ,PIP,TZP,CN, AN,NET

Legends: A. Main hospital, B. Municipal hospital, C. Outpatients´ department. 1. Obstetric-gynaecological ward, 2. Orthopaedic-traumatical ward, 3. Orthopaedic ward, 4. Infectious ward, 5. Urologic ward, 6. Rehabilitation ward, 7. Intensive Care Unit ward (ICU), 8. Paediatric ward, 9. Surgical ward, 10. Neurological ward, 11. Pathology of pregnancy ward, 12. Oncology ward.

DISCUSSION

The hospital environment remarkably promotes selection and quick distribution of resistant strains. One of the essential steps leading to a reduction of nosocomial infections is a constant monitoring of etiological agents and resistance of intrahospital strains. It is of crucial importance to carry out epidemiological surveys including a detailed characteristic and relationship among strains isolated in particular environment and time, as well as to become aware of risk factors, sources and ways of infection distribution (1, 8, 9, 13). To obtain reliable results the application of molecular methods seems to be inevitable.

To differentiate precisely among *P. aeruginosa* isolated from two hospitals in Siedlce (Poland), BOX-PCR typing was carried out. PCR fingerprinting has shown 38 genetic patterns, among them 7 main genotypes consisting of 3 to 8 strains and 31 other unique patterns. High number of genotypic patterns pointed to marked intrahospital differentiation of *P. aeruginosa* strains that are widely distributed in nature, especially in humid environments. It indicated various sources of strains and their constant exchange. Some strains were generally resistant to tested antibiotics, what confirmed the development of secondary resistance and their intrahospital selection. Based on dates of strain isolation, and their resistance to antibiotics, it is highly probable that selection of highly resistant isolates takes place in ICU, Urologic and Orthopedic wards where *P. aeruginosa* is one of the most frequent and severe causes of infection, especially in patients with respiratory, urinary and wound infections. Several studies have demonstrated associations with a source of *P. aeruginosa* infection and antibiotic resistance (1, 5, 18, 24). The other strains of genotypes, especially those, which consisted of strains from faeces (serotype O6) taken from patients hospitalized in the Infectious Ward of the Municipal Hospital and the Paediatric Ward of the Main Hospital, frequently expressed susceptibility to tested antimicrobial agents. This proved incidence of exogenous strains entering the hospital environment. Some of the numerous genotypes were distributed in one, or more than one unit. This may indicate that cross contamination among patients lead to the spread of these genotypes among the various units, possibly through transient hand carriage by health care personnel due to contact with contaminated surfaces, or by patient contact with contaminated surfaces or medical equipment (19). The incidence of the same genotypes of *P. aeruginosa* in two different hospitals drew attention to a possibility of a long-distance strain transmission, which might be linked to the movement of patients, visitors, medical and paramedical staff. The importance of cross acquisition in the epidemiology of nosocomial colonization and infection with *P. aerugionsa* was reported by others (1, 8, 25). Fiett *et al.* (8) demonstrated clonal relations within populations of *P. aeruginosa* strains isolated in four different hospitals in Poland. Bergmans *et al.* (1) who studied 100 patients admitted to an ICU ward showed that cross colonization accounted for 50% of all cases of acquired *P. aeruginosa* colonization, and the rest of 50% of patients were probably colonized from endogenous sources. Cross transmission and treatment failure were also the two main problems at Turkish medical centers (25).

This study demonstrated that BOX-PCR is a rapid, highly discriminatory and reproducible assay that proved to be powerful surveillance tools for typing as well as characterizing clinical *P. aeruginosa* isolates. This is in agreement with the studies of Syrmis *et al.* (22), in which the BOX-PCR method showed the high discriminatory power. These authors reported six major clonal groups, and 58 distinct clonal groups among 163 *P. aeruginsa* strains isolated from patients with *cystic fibrosis*.

P. aeruginosa strains were also verified by classical typing techniques. The studied strains showed poor differentiation of phenotypic features, especially such as: serotypes and types of growth on cetrimide agar. The total of 62 *P. aeruginosa* strains were classified into 9 different serotypes. Most of them (69.3%) belonged to O6 serotype, secondly to serotype O1 (9.7%) (the dominant type among MDR strains). The observed strains demonstrated 5 types of growth on cetrimide agar medium. The celadon type appeared most frequently (61.3%) whereas the green or green-yellow types were rarer (17.7 and

11.3%). The frequency of distribution of the O antigen types differs considerably in various publications. Czekajło-Kołodziej *et. al*. (4) demonstrated among over 50% of clinical *P. aeruginosa* strains isolated from the lower respiratory tract of patients admitted to ICU the production of green-yellow colonies, typing by O11 sera, and resistance to many antibiotics. Muller-Premru and Gubina (15) observed two O serotypes 11 and 6 to be prevalent (36% and 14.4% respectively) among clinical isolates. Antibiotic resistance of strains was higher in serotype O11 than in serotype O6. In a study of 73 *P. aeruginosa* strains from various clinical and environmental sources, Pirnay *et al*. (17) reported the predominant serotypes to be O11 (15.1%), O1 (12.3%), O6 (10.9%) and O12 (9.6%). Amongst 48 AFLP (amplified fragment length polymorphism) types isolated from burns patients, 58.3% were reported as serotypes O1, O6, O11 or O12 (19). In a survey of 92 genetically distinct bacteraemia isolates, O6 (25.0%) and O11 (18.0%) were reported to be the most common serotypes (2). In a study of 23 isolates from contact lens wearers, Thuruthyil *et al*. (23) reported O1 (30.0%), O6 (17.0%) and O11 (17.0%) as the most common serotypes.

In conclusion, among all used methods in this work BOX-PCR turned out to be a powerful tool for the study of clinical *P. aeruginosa* isolates diversity. However, we suggest that maximum discrimination can be best achieved by a combination of phenotypic and genotypic methods.

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