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Pseudomonas Aeruginosa in Water of Hamam or Turkish Bath: Serotyping and Antibiotic Susceptibility

[1,2]*Abdelmoula El Ouardi,* [1]*Samira Senouci,* [1,2]*Fatima El Habib and* [2]*My Mustapha Ennaji*

[1]Department of Microbiology and Food Hygiene, National Institute of Hygiene,
Rabat, 27 Avenue Ibn Battuta, BP. 769 Rabat Morocco
[2]Laboratory Virology, Hygiene and Microbiology, Faculty of Sciences and Techniques Mohammedia,
University Hassan II Mohammedia-Casablanca

Abstract: *Pseudomonas aeruginosa* is an opportunistic pathogen which is found ubiquitously in water and soil environments. This bacterium easily adapts to its environment by developing variants that show increased resistance towards the antimicrobial treatment. The results of our study on a large number of cold water samples from the wells of bath confirmed the existence of frequent *P. aeruginosa* colonization (57% of examining samples). Among the 321 strains of P. aeruginosas which benefits from a serotyping, the six most frequent serotypes were O1 (29.9%), O4 (15.0%), O5 (11.5%), O6 (7.2 %), O2 (5.9%) and O16 (5%). The medium antibiotic susceptibility included: ticarcillin (61 %), ticarcillin-clavulanic acid (67 %), piperacillin (78 %), piperacillin-tazobactam (82 %); aztreonam (63 %); imipenem (88 %); Cefotaxime (76 %), ceftazidime (76 %), cefepime (68 %), cefpirome (47 %); gentamicin (71 %), amikacin (70 %), netilmicin (74 %), tobramycin (76 %); ciprofloxacin (66 %) and finally colistin (79 %). Our percentage of resistant strains to cefepime and ticarcillin was high.

Key words: Antibiotic Susceptibility · Bath · *Pseudomonas Aeruginosa* · Wells

INTRODUCTION

Steam bath, Turkish bath, bath Moor or Hamam is an integral part of community life. All classes of society frequent this public place (40-200 bathers per day), spite the growth of modernization programs and home bathrooms.

Recreational waters including the waters of Hamam are monitored worldwide to protect the health of bathers against infection with *Pseudomonas* or enteric pathogens. Indeed, the microbial quality of bathing water can change rapidly within a 24 h period [1]. Among these microorganisms, *P. aeruginosa* can nest by forming the resistant biofilms [2, 3]. Cells released from biofilms contaminate the water phase and pose a potential threat to human health, especially when occurring in man-made water systems or in technical water systems (drinking water distribution systems, plumbing systems, public swimming pools, Hamams and industrial water systems) [4]. Once developed, their biofilms are harder to be eradicated and may serve as a chronic source of microbial contamination [5, 6]. In fact, potential pathogens are protected from external stresses, such as the action of disinfectants and can persist and multiply [7]. Water temperature, flow, stagnation, pipe materials, degree of pipe corrosion, high water shear stress and flushing are well known factors favoring *P. aeruginosa* growth [8]. This bacterium has minimal nutritional requirements and can tolerate a wide variety of physical conditions [9].

P. aeruginosa is intrinsically resistant to several classes of antibiotics, thus limiting therapeutic options. Unfortunately, antimicrobial therapy is becoming even more problematic due to acquired or mutational resistance [10]. A wide range of resistance mechanisms have been identified including multi-drug efflux pumps

Corresponding Author: Abdelmoula El Ouardi, Department of Microbiology and Food Hygiene, Laboratory Virology, Hygiene and Microbiology, Faculty of Sciences and Techniques Mohammedia, National Institute of Hygiene, Rabat, 27 Avenue Ibn Battuta, BP. 769 Rabat Morocco, University Hassan II Mohammedia-Casablanca.

(which can confer resistance to, among others, cephalosporins, ureidopenicillins, fluoroquinolones and aminoglycosides), aminoglycoside-modifying enzymes, b-lactamases and target site modifications [11].

Acquired resistance in *P. aeruginosa* is caused by one or more of several mechanisms: (i) mutational modification of antibiotic targets, such as gyrase, topoisomerase or ribosomal proteins, which confer resistance to fluoroquinolones or aminoglycosides, respectively; (ii) mutational derepression of the chromosomally coded AmpC beta-lactamase; (iii) mutational loss of outer membrane proteins preventing the uptake of antimicrobial substances such as carbapenems; (iv) mutational upregulation of efflux systems, that can confer resistance to beta-lactams, fluoroquinolones and aminoglycosides; finally (v) acquisition of plasmid-mediated resistance genes coding for various beta-lactamases and aminoglycoside modifying enzymes that can confer resistance to various beta-lactams including carbapenems and aminoglycosides [10]. Bacillus pyocyaneus is a life-threatening multidrug resistant (MDR) [12-14]. Furthermore, increasing trends of MDR *P. aeruginosa* in the Morocco have been reported [15]. In addition there is limited published data about the prevalence of this pathogen in baths publics [16]. Therefore, the aim of this study was to identify the strains of *P. aeruginosa*, well as their antimicrobial susceptibilities, in bathroom water taps of Hamam over the seasons.

MATERIALS AND METHODS

Study Setting and Sampling: During the four seasons of the year 2011, In Rabat capital of Morocco, 508 cold water samples were collected from 71 Hamams of the popular districts. All water samples are collected at the tap of Hamam served by wells.

Isolation and Identification of *P. aeruginosa* Strain: *P. aeruginosa* was isolated and characterized from water specimens according to the official Morocco method [17]: aliquots of 100 ml were filtered through a 0.45 µm gridded cellulose nitrate membrane (Sortorius) and the membrane was placed on Cetrimid Agar (*Pseudomonas* CFC agar, Oxoid CM559) with 10 ml Glycerol/l, incubated at 42 °C for 48 h. Colonies that clearly showed pyocyanin production (blue green colonies and fluorescence at 360 nm) were considered positive for *P. aeruginosa*. All colonies were confirmed using King P Agar (Scharleau) and King F Agar (Scharleau) incubated at 37 °C for 24 h and on testing were found to be oxidase positive (Merck-testswobs).

Serotyping: All *P. aeruginosa* isolates were serotyped by the slide agglutination technique according to the manufacturer's specifications (Biorad, Marnes-la-Coquette, France). Serotyping was performed with specific polyclonal antisera to 17 somatic O antigens based on the International Antigenic Typing Scheme [18] purchased from Biorad. Briefly, the slide test was conducted with four individual polyvalent mixtures antisera, using living suspensions of the isolates as test antigens from fresh blood agar cultured for 18 to 24 h. If it was positive in one of the pools, we then proceeded with monovalent grouping serum.

Antimicrobial Susceptibility Testing: The antimicrobial susceptibilities of all bacterial strains were determined using the standard Kirby-Bauer disk-diffusion method for 16 antibiotics, according to the Clinical and Laboratory Standards Institute breakpoints [19]. The antibiotics included ticarcillin (75 µg), ticarcillin-clavulanic acid (75 µg/10 µg), piperacillin (75 µg), piperacillin-tazobactam (100 µg/10 µg), aztreonam (30 µg), imipenem (10 µg), Cefotaxime (75 µg), ceftazidime (30 µg), cefepime (30 µg), cefpirome (30 µg), gentamicin (30 µg), amikacin (30 µg), netilmicin (75 µg), tobramycin (30 µg), ciprofloxacin (5 µg) and colistin (50 µg). The strains with intermediate susceptibility were considered resistant. *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used for quality control.

RESULTS

Frequency of Different Serotypes of *P. aeruginosa:* Among the 321 strains of *P. aeruginosa* (57% of examining samples) the six most frequent serotypes were O1 (29.9%), O4 (15.0%), O5 (11.5%), O6 (7.2 %), O2 (5.9%) and O16 (5%). Other serotypes were encountered rarely, with a frequency strictly less than 5% (Table 1).

Antibiotic Susceptibility of *P. aeruginosa* Isolates: The medium susceptibility was given by Penicillins: ticarcillin (61 %), ticarcillin-clavulanic acid (67 %), piperacillin (78 %), piperacillin-tazobactam (82 %), Monobactams: aztreonam (63 %), Carbapenems: imipenem (88 %), Cephalosporin: Cefotaxime (76 %), ceftazidime (76 %), cefepime (68 %), cefpirome (47 %), Aminosides: gentamicin (71 %), amikacin (70 %), netilmicin (74 %), tobramycin (76 %), Fluoroquinolone: ciprofloxacin (66 %) finally Lipopeptides (polymyxins): colistin (79 %) (Figure 1).

Table 1: Antibiotic susceptibility of *Pseudomonas aeruginosa* different serotypes (321 strains)

Serotypes	Number of strains	Frequency (%)	TIC	TCC	PIP	TZP	CAZ	FEP	CPO	CTX	GM	AN	NET	TM	ATM	CIP	IPM	CS
O:1	96	29,9	73	73	85	80	76	69	53	66	64	71	73	80	74	76	86	88
O:4	48	15,0	52	60	79	82	73	64	37	70	66	78	75	79	56	52	84	84
O:5	37	11,5	60	68	83	84	80	74	40	62	60	86	79	84	57	83	80	85
O:6	23	7,2	68	76	82	80	79	69	50	65	64	68	73	80	65	72	87	78
O:2	19	5,9	69	78	90	84	87	82	36	80	79	82	70	83	67	51	82	76
O:16	16	5,0	61	65	78	74	80	65	42	90	85	92	94	89	62	59	92	67
O:15	14	4,4	63	67	80	76	80	63	50	91	81	88	90	87	66	60	90	85
O:8	13	4,1	77	80	93	88	93	90	70	90	94	93	96	95	77	87	97	85
O:10	13	4,1	71	86	88	90	89	82	67	84	91	86	80	82	76	89	97	79
O:3	12	3,7	63	69	85	89	79	68	41	89	87	85	85	86	63	76	95	72
O:11	12	3,7	49	50	61	80	63	50	36	78	72	75	76	71	54	53	80	73
O:7	8	2,5	67	78	79	95	83	75	58	86	60	54	54	52	67	75	96	86
O:12	6	1,9	12	10	24	60	59	23	15	20	30	25	28	30	22	18	75	64
O:13	4	1,3	67	74	78	90	84	74	60	91	64	59	56	53	70	76	91	80

TIC: ticarcillin; TCC: ticarcillin-clavulanic acid; PIP: piperacillin; TZP: piperacillin-tazobactam; CAZ: ceftazidime; FEP: cefepime; CPO: cefpirome; CTX: cefotaxime; GM: gentamicin; AN: amikacin; NET: netilmicin; TM: tobramycin; ATM: aztreonam; CIP: ciprofloxacin; IPM: imipenem; CS: colistin.

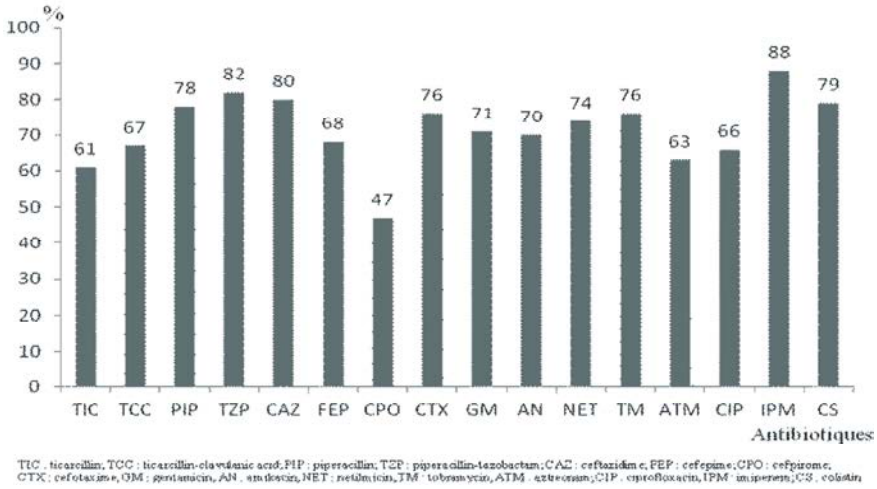

Fig. 1: *P. aeruginosa*: overall percentage of strains susceptible to the antibiotics tested

Sensitivity by Serotype: O1 and O6 were the most sensitive of six common serotypes (O1, O4, O5, O6, O16 and O2), while serotype O12 had the lowest rates of susceptibility (Table 1).

DISCUSSION

The results of our study on a large number of cold water samples confirm the existence of frequent *P. aeruginosa* colonization (57% of examining samples).

Despite disinfection, persistence of contamination throughout the year was explained by: Firstly, 95 % of Hamam were served by treated water from wells. Secondly, inadequate disinfection procedures in these systems water, in fact, high-temperature flushing or chlorination may also be appropriate strategies to decrease potentially high concentrations of waterborne organisms [20]. However for majority Hamams analyzed, they were satisfied at disinfection chemical, insufficient alone to eradicate bacteria from water systems. Furthermore the type and dosage of a chemical disinfectant needed to be selected carefully because each situation is different in terms of background conditions (e.g., pH, temperature and levels of organic and inorganic constituents that may affect the effectiveness of a disinfectant) [21]. When thermal shock treatment is not possible, shock chlorination may provide an alternative. Experience with this method of decontamination is limited, however and high levels of free chlorine can corrode metals. Chlorine should be added, preferably overnight, to achieve a free chlorine residual of at least 2 mg/L (2 ppm) throughout the system. In effect, biofilms or microcolonies provide sites with favorable conditions by protecting the pathogens from high chlorine concentrations and shear stress and providing better nutrient conditions [22]. The ability of *P. aeruginosa* to persist in environmental niches such as pipes and taps is important [23]. When growing as a complex mass of cells

attached to a surface, *P. aeruginosa* cells can be significantly more resistant to biocides than when they are in a planktonic (free-floating) [24]. *P. aeruginosa* can survive for long periods in biofilm. Likewise, the old water lines and the stagnation of water lines in these Hamams are risk factors for breeding biofilms, so it is important to continuously clean up the water lines. It is estimated that 95% of the overall biomass at water distribution systems is located on the inner surface of the pipe walls as a biofilm, while only 5% occurs in the water phase [25].

Third, irregular control and the absence of regulation, in fact the control of biofilm-associated *P. aeruginosa* may lead to the most effective control measure in preventing *P. aeruginosa* pathogenesis. Thus, the control of microbial growth is required in many microbiologically sensitive environments, where wet or moist surfaces provide favorable conditions for microbial proliferation and biofilm formation [26]. Biofilm control methods must take into account the knowledge of the constitutive microflora and their responsive behavior to control. Thereby, the study of biofilm ecology and interactions might help to improve our understanding of their resistance mechanisms to control strategies [27, 28].

P. aeruginosa shows a particular propensity for the development of resistance, which limits future therapeutic choices and is associated with increased mortality rates and higher costs [29]. The overuse and misuse of antibiotics have also led to the selection of resistant strains against which very few therapeutic options exist [30]. Overall susceptibility to ticarcillin was 61%. The global sensibility rate to piperacillin, piperacillin/tazobactarn, Ticarcillin-clavulanic acid, cefepime, ceftazidime, cefotaxime, cefpirome, imipenem, colistin and aztreonam are equivalent, respectively 78, 82, 67, 68, 76, 76, 47, 79 and 63%, respectively. In contrast, resistance levels vary: they were lower with imipenem (12%), piperacillin / tazobactam (18%), colistin (21%) and piperacillin (22%); they were higher with cefpirome (53%), ticarcillin (39%), aztreonam (37%) and cefepime (32%). Cefepime and cefpirome fourth-generation cephalosphorin, were two of the few agents remaining that having reliable activity against *P. aeruginosa*. However, increased prevalence of resistance to cefepime among these organisms has been noted [31]. Fluoroquinolones and aminoglycosides are two important classes of antibiotics used in the treatment of *Pseudomonas* infections. The pyocyanique from our study were often more susceptible to tobramycin (76%) than netilmicin (74%), gentamicin (71%) and amikacin (70%). Fluoroquinolone resistance in *P. aeruginosa* was believed to be largely due to the selection of organisms with point mutations in the topoisomerase enzymes that are targets of fluoroquinolones [32], ciprofloxacin maintains moderate activity about the strains studied.

Serotypes O: 12 and O: 11 are most commonly associated with multidrug resistance [33]. It can be seen from table 1, a significant level of resistance with serotype O12, classically associated with highest resistance rates.

In this study of 71 Hamams in Rabat, 77% of the water systems were found to be colonized with *P. aeruginosa*. A fast and reliable screening tool for this bacterium is necessary for limiting the impact of a contamination.

This pathogen provides impressive chromosomally-encoded mechanisms of intrinsic resistance, as well as the potential to mutate, gaining resistance to current antibiotics. Our percentage of strains CPO (R) and TIC (R) respectively 53% and 49% is high (Figure 1) is high. Interest epidemiological of serogrouping by major agglutination antigens O is now well known. In addition, our study confirmed the particularity of resistance profiles of serotype O12, which have to be controlled very carefully. We believe current recommendations to avoid public baths (Hamam) and to avoid drinking running tap water are inappropriate, as long as the water is well maintained according to local hygiene guidelines.

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