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RESEARCH ARTICLE

Prevalence and evaluation of resistance to antibiotics of genera *Proteus, Morganella* and *Providencia* isolates in University Hospital of Constantine, Algeria

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Abstract

The three genera *Proteus*, *Morganella* and *Providencia (PMP)* are opportunistic microorganisms which cause serious hospital and community infections. However, in many laboratories in developing countries, differentiation of these genera into species is not generally done during bacteriological diagnosis due to high cost and special skills involved. This study aimed at determining the occurrence and antimicrobial susceptibility of genera *PMP* isolated from different clinical samples in Constantine, Algeria. 17 834 clinical samples were collected in the Clinical Microbiology Laboratory of Benbadis University Hospital in Constantine, during the year 2011. The isolates were identified to the species level using conventional biochemical tests and the identification was confirmed on the basis of the results of Analytical Profile Index API20E tests. Antimicrobial susceptibility of isolates was determined by the disk-diffusion method on Mueller-Hinton Agar. 4173 were positive (23.40% of total samples). Four *PMP* species were recovered from 304 of 4173 positive result giving 7.28 % prevalence of *PMP* infections mainly isolates from in-patients (78.95%);
Proteus mirabilis was the commonest (70.06 %), followed by *Proteus vulgaris* (15.13 %). *Morganella morganii* accounted for (10.19 %) and *Providencia stuartii* (4.60 %). Strains of *PMP* most commonly isolated from pus (47.04 %) followed by urine samples. All of *PMP* strains isolated were susceptible to carbapenems. We reported that strains were generally susceptible to Cefoxitin, Cefotaxime, Imipenem, Amikacin, Ciprofloxacin. We observed a high percentage of *PMP* strains resistant to Amoxicillin, Nalidixic acid, Triméthoprim/ sulfamethoxazol. However, *M. morganii* and *P. stuartii* isolates exhibited a high resistance to Ticarcillin, Gentamicin, Chloramphénicol. More than 61% of all strains were found to be multiple drugs resistant. Extended Spectrum β-Lactamases (ESBL) were produced by 10.33 - 16.13 % of *PMP* strains.

Introduction

The three genera *Proteus*, *Morganella* and *Providencia* (*PMP*) belongs to the *Enterobacteriaceae* family comprising approximately a total of ten species (Manos and Belas, 2006). The proximity of these three genera is observed on phenotypic studies and also genetically, as shown in phylogenetic trees constructed on the basis of RNA sequences and gene *gyrB* (Freney et al., 2007;Dauga, 2002). Due to close similarity of *Proteus*, *Morganella*, and *Providencia* rods, all three genera were placed in the tribe *Proteeae*, however, the tribe designation is not often used (Rózalski and Staczek, 2009). Members of these three genera are components of the normal bacterial flora of the intestinal

tracts of humans and animals and are widespread in the environment. Some species belonging to these genera have been recognized as opportunistic pathogens, causing primary and secondary infections (O'Hara et al., 2000). Interest in the species comprised in these genera has occurred mainly from a clinical perspective, as they include a number of significant human pathogens. These pathogens have a diverse mode of transmission, and hence can cause infection in different anatomical sites of the body. Some of the sources of transmission are soil, contaminated water, food, equipment, intravenous solutions, the hands of patients and healthcare personnel (Feglo et al., 2010). Most infections are associated with prolonged hospitalization and in the case of *Proteus* and *Morganella* spp., associated with colonization of indwelling catheters and urinary tract infections (Manos and Belas, 2006). This study seeks to evaluate the frequency of isolation and antibiotic susceptibility of *Proteus, Morganella* and *Providencia* recovered from infections in patients hospitalized and outpatients in the University Hospital of Constantine in Algeria.

Materials and Methods

Isolation site

The study was undertaken in the Laboratory of clinical bacteriology at the University Hospital of Constantine, Algeria, during the year 2011. A large variety of clinical samples were issued: urine, purulent material from wounds or abscesses, blood or aspirates (of joint fluid, pleural fluid, ascitic fluid and pus) and other diverse material (such as catheter) collected from 17834 patients suspected of bacterial infection. Demographic data (such as sex, in-patient and out-patient status) of the patients were recorded prior to sample collection.

Culture and Identification

All samples collected were aseptically inoculated on the various media included: Blood, Chocolate, Nutrient and Hektoen agars, and incubated at 37°C for 24 hours. However, suspected *PMP* colonies were isolated from simple media (Nutrient or Hektoen agars)

The morphological characteristics of the colonies including sizes, shapes, ability to swarm, ability or inability to ferment lactose, odor and microscopic features of the cells were recorded. Identification of clinical isolates was carried out through biochemical tests according to Bergey's Manual of Systematic Bacteriology (Holt and Williams, 1989). The identification of selected *Proteus, Morganella* and *Providencia* colonies was confirmed by Analytical Profile Index; API20E (BioMerieux, Marcy l'Etoile, France)

Antimicrobial susceptibility test

Susceptibility test was conducted by the disc diffusion method on Mueller-Hinton. The different antimicrobial agents used and their disc concentration were : amoxicillin (25 µg), amoxicillin/clavulanic acid (20/10 µg), ticarcillin (75 µg), cefazolin (30 µg), cefoxitin (30 µg), cefotaxime (30 µg), imipenem (10 µg), gentamicin (15 µg (10 UI), amikacin (30 µg), colistin (50 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), trimethoprim/sulfamethoxazol (1,25/23,75 µg), chloramphenicol (30 µg). Plates were incubated for 24 h at 37°C after which the inhibition zones were measured. The results were interpreted according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute (CLSI), 2007). The isolates that showed resistance to 3 or more antibacterial agents from different classes were regarded as MDR and were included in this study.

Detection of β- Lactamases

ESBL producing isolates were detected by double disc synergy test including amoxicillin/clavulanic acid (AMC) containing disc placed in the center, 30 mm distant from–cefotaxime (CTX), ceftriaxone (CRO) or ceftazidime (CAZ) containing dics. Production of ESBL was indicated by the synergy between the CAZ, CTX and CRO and AMX/CLA disc.

Results

Proteus, Morganella and *Providencia* species isolated

Among the 17834 samples collected, 4173 were positive and represented 23.39% of total samples, 1585 were contaminated and represented 8.88% of total samples. The remaining samples were negative and represented 67.86 % which corresponded to 12076 samples. Four species were recovered from 304 of the 4173 positive results, rendering a prevalence rate of 7.28 %. *PMP* were isolated from nearly 1.7% of total samples collected / year. *Proteus mirabilis* was the most prevalent (70.06 %), followed by *Proteus vulgaris* (15.13 %). *Morganella morganii*

accounted for (10.19 %) and *Providencia stuartii* (4.60 %) of the *Proteus, Morganella* and *Providencia* isolates (Figure 1).

The *PMP* isolates were obtained from 14 different specimens as indicated on* (Table 1) with the following percentage representations: pus (47.04 %), urine (31.91 %), blood (5.92 %), sonde (5.92 %), tracheal (2.63 %), catheter (1.64 %), Ascitic fluid (0.98 %), peritoneal fluid (0.98 %), pleural fluid (0.65 %), vaginal sample (0.65 %), gastric fluid (0.33 %), cerebrospinal fluid (0.33 %), mammary flow (0.65 %) and umbilical cord (0.33 %). *P. mirabilis* was the most frequent species isolated in all the specimens with the exception of catheter, from which there were recovered 2 *P. mirabilis* isolates respect 3 isolates of *P. stuartii*, and cerebrospinal fluid from which it was only isolated 1 *P. vulgaris. P. mirabilis* was isolated from pus (46.01%), urine (33.33%), sonde (6.10%) and blood (5.63%). *P. vulgaris* was highly isolated from pus (67.39%) followed by urine (17.39). *M. morganii* was more frequent in urine (48.39%) and *P. stuartii* in pus (35.71%).

There were a total of 240 samples from in-patients (78.95%) and 64 out-patients (21.05%) as shown in Table 1 below. A total of 161 *P. mirabilis* (75.59 %) were isolated from in-patients whereas 52 (24.41 %) were from out-patients. On the other hand *P. vulgaris* was isolated from in-patients in 42 (91.30 %) cases and 4 (8.70 %) from out-patients. Also, 24 (77.42 %) *M. morganii* were isolated from in-patients and 7 (22.58%) from out-patients. Finally, all but 1 *P. stuartii* isolates were recovered from in-patients. The most frequent positive sample from in patients corresponded to pus (64.71 %). Urine samples corresponded to 48% from in patients and 49% from outpatients.

There were a total of 168 (55.26 %) isolates recovered from males and 136 (44.73 %) from females. As presented in (Table 1), *P. mirabilis* infection was detected among 109 males (51.17%) and 104 females (48.83%). *P. vulgaris*, on the other hand, was isolated from 31 males (67.39 %) and 15 females (32. 61%). *M. morganii* was isolated from 17 males (54.84 %) and 14 females (45.16 %), and finally, *P. stuartii* was isolated from 11 males (78.57 %) and 3 females (21.43 %).

Antimicrobial susceptibility of *Proteus, Morganella* and *Providencia* isolates

A review of the antimicrobial resistance profile of isolates from the different clinical specimens showed that cefoxitin, cefotaxime, imipenem, amikacin, ciprofloxacin were the most active antibiotics. Antibiotics with the lowest activities on all four species were amoxicillin, nalidixic acid, trimethoprim + sulfamethoxazol (resistance 42.62 % - 100 %). However, *M. morganii* and *P. stuartii* isolates exhibited a high resistance to ticarcillin, gentamicin, chloramphenicol. More than 61% of all isolates were found to be multiple drug resistant (resistant to at least 3 antibiotics). However, 10.33 - 16.13 % produced ESBL. There was no significant difference in the ESBL production by the various species (table 2).

Figure 1. Distribution of the various *Proteus, Morganella and Providencia* species isolated

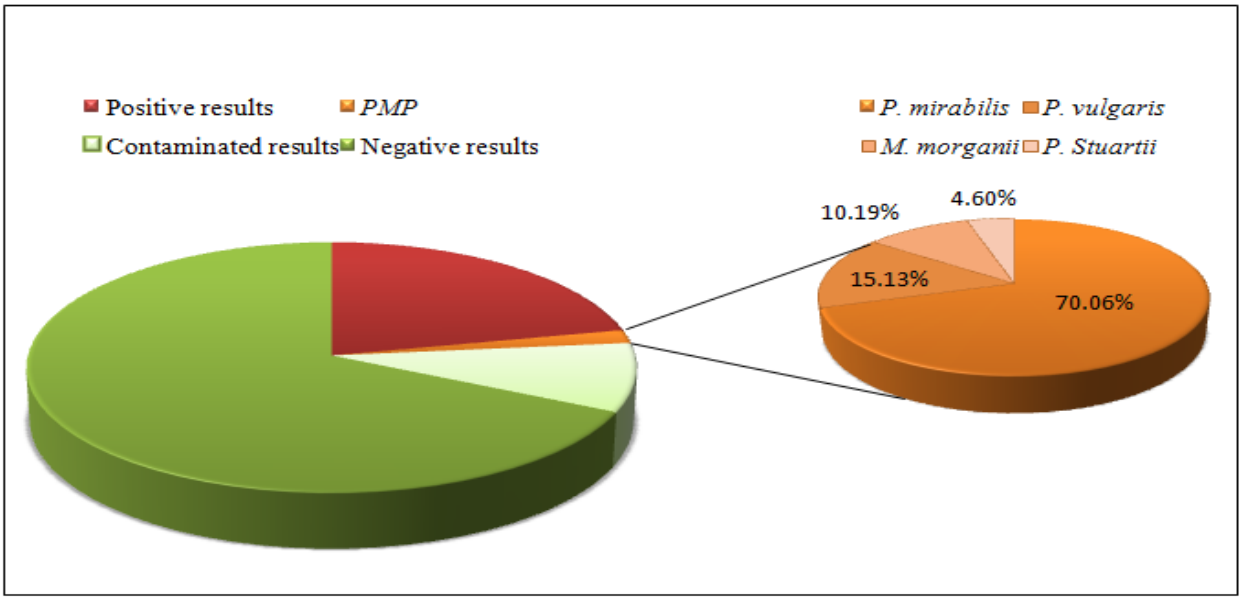

Table 1. *Proteus, Morganella and Providencia* species isolated from various specimens in relation to in-patients and out-patients

Specimens	No	Species																			
		P. mirabilis					*P. vulgaris*					*M. morganii*					*P. stuartii*				
		In-patient		Out-patient		Total	In-patient		Out-patient		Total	In-patient		Out-patient		Total	In-patient		Out-patient		Total
		♂	♀	♂	♀		♂	♀	♂	♀		♂	♀	♂	♀		♂	♀	♂	♀	
Pus	143	56	33	4	5	98	20	9	0	2	31	8	1	0	0	9	4	1	0	0	5
Urine	97	10	22	17	22	71	5	1	2	0	8	2	6	3	4	15	2	0	0	1	3
Blood	18	5	7	0	0	12	3	1	0	0	4	0	1	0	0	1	1	0	0	0	1
Sonde	18	8	5	0	0	13	1	0	0	0	1	2	2	0	0	4	0	0	0	0	0
Tracheal	8	3	1	0	0	4	0	0	0	0	0	2	0	0	0	2	1	1	0	0	2
Catheter	5	0	2	0	0	2	0	0	0	0	0	0	0	0	0	0	3	0	0	0	3
Ascitic fluid	3	0	1	1	0	2	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
Peritoneal fluid	3	2	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pleural fluid	2	1	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vaginal sample	2	0	1	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mammary Flow	2	0	1	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gastric fluid	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cerebrospinal fluid	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
Umbilical cord	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	304	87	74	22	30	213	29	13	2	2	46	14	10	3	4	31	11	2	0	1	14
		161		52			42		4			24		7			13		1		

Table 2. Percentage of antibiotic resistance of *Proteus, Morganella* and *Providencia* isolates

	AMX	AMC	TIC	CZO	FOX	CTX	IMP	GEN	AMK	COL	NAL	CIP	SXT	CHL	MDR	ESBL
P.mirabilis	60.80	32.87	54.17	39.41	3.29	18.46	0	20.90	17.53	*	42.62	24.86	55.74	44.19	61.03	10.33
P. vulgaris	*	41.38	35	*	2.17	19,05	0	32.5	12.82	*	45.95	26.67	60.53	34.29	67.39	10.87
M. morganii	*	*	52	*	52	37.93	0	50	23.08	*	62.96	36	72	64.29	87.09	16.13
P. stuartii	*	*	80	*	33.33	54.55	0	72.73	75	*	45.45	27.27	100	88.89	92.86	14.29

AMX: amoxicillin; AMC : amoxicillin + clavulanic acid; TIC: ticarcillin; CZO: cefazolin; FOX : cefoxitin; CTX : cefotaxime; IMP : imipenem; GEN : gentamicin; AMK : amikacin; COL : colistin; NAL : nalidixic acid; CIP : ciprofloxacin; SXT : trimethoprim + sulfamethoxazol; CHL : chloramphenicol; MDR: Multi-drug resistance; ESBL: Extended-spectrum beta-lactamase. * : Natural Resistance

Discussion

Species identification is critical in the diagnosis and treatment of people infected with *PMP*. It is also required in disease prevention, patient management, and surveillance of infection. While antimicrobial resistance surveys have revealed that members of the *PMP* are relatively frequent among clinical isolates, and a number of series have investigated specific subsets of infections caused by these organisms (Mahamat et al., 2006;Zalas-Wiecek et al., 2012;Shima et al., 2012), few studies have focused on the *PMP* as a whole (Kim et al., 2003). This study represents the first population-based description of *PMP* group in Algeria, and provides new information on the distribution of clinical isolates at the University Hospital of Constantine, Algeria. *PMP* were isolated from nearly 1.7% of samples collected in this study, confirming that these organisms are relatively frequent. Four *PMP* species (*P.mirabilis*, *P. vulgaris*, *M. morganii* and *P. stuartii*) were identified to be responsible for causing infections in various anatomical sites. *P. mirabilis* was the most frequent species isolated (70.06 %), hence responsible for the majority of *PMP* infections and therefore supported by the fact that it is the most virulent of all the *PMP* (O'Hara et al., 2000;Coker et al., 2000), and hence the commonest etiologic agent of both community and hospital acquired infections. This can be explained by the high carrier rate (25%) in the human digestive tract (Farmer, 1999). The intestines are although the major reservoir of these bacteria in humans, resulting from auto-infection or transmission from patient to patient in hospitals (Farmer, 1999). The findings of our study are in agreement with those of some previous studies in Germany (Muller, 1986), Trinidad (Orrett, 1999), England, Wales, Northern Ireland (CDR Weekly, 2006) and Ghana (Feglo et al., 2010). However, the study of Kim et *al*. in Korea showed that *M. morganii* are the most common species isolated (61 cases), followed by *P. mirabilis* and *P. vulgaris*. However, this study was focused solely on isolates from bacteraemia (Kim et al., 2003). Pus recorded the highest percentage of *Proteus* isolates (46.0%) followed by urine samples (33,33%) and this confirmed the findings of Newman et *al.* (Newman et al., 2006) and Feglo et *al.* (Feglo et al., 2010) in Ghana and Yah et *al*. (Yah et al., 2007) in Nigeria. *Proteus* is therefore a common cause of wound infections in Algeria. Our findings are, however, in contrast with those of Reslinski et *al.* (Reslinski et al., 2005) and Orrett (Orrett, 1999) from Europe and Asia which showed *Proteus* species to be more commonly encountered in urine than in other clinical specimens*.

PMP infections were also common among the in-patients (78.95%) as compared to out-patients (21.05%). This trend can be explained by the fact that *PMP* behave as opportunistic pathogens and are therefore involved in infectious diseases, especially among individuals hospitalized, immunocompromised, paraplegic or with urinary tract abnormalities (Mahamat et al., 2006;O'Hara et al., 2000). In the United States *P. mirabilis* accounts for approximately 3% of nosocomial infections (Centers for Disease Control and Prevention, 1996). There was no significant difference ($p > 0.05$) between the males and females infected with *PMP* in this study.

The *PMP* species displayed high antimicrobial resistance rates. similar to previous reports in Algeria (Rahal, 2008). The pattern of resistance to antibiotics of *PMP* strains seems to be correlated to some extent with their classification into four species.

More than 61% of the isolates were found to be multiple drug resistant. The high antibiotic resistance of *PMP* may be an indication of the resistance levels among the *Enterobacteriaceae* since indiscriminate consumption of antibiotics provides selective pressure, leading to a higher prevalence of resistant bacteria (Levy, 1999), which is very common in developing countries like Algeria. Not only, these species are potential causes of infections but also potential reservoirs of resistance genes that could be transferred to other bacterial pathogens. The frequency of ESBL-producing strains is comparable to that reported by Rahal et *al*. (Rahal, 2008) in Algeria. The high level of β-lactamase producing isolates and multi-drug resistant isolates denotes an increase in the resistance menace. This general situation is the result of selection pressure due to extensive use of β-lactam antibiotics. In addition, the acquired resistance due to their plasmid determinism has great power to spread.

Conclusion

Resistance to antibiotics is an increasingly common problem in both veterinary and human medicine, and its management is a subject of urgent debate. Species identification and a better study of the epidemiology of antimicrobial resistance will improve the management and control of infections and the therapeutic management of patients while reducing the prescription of large spectrum antibiotics. This can only be done at the expense of better monitoring. These practices are generally absent in most of our hospitals, mainly due to the high costs involved. Therefore this study is a step towards the generation of national data on the prevalence of antimicrobial resistant pathogens in Algeria.

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