

Original Article

Antibiotic resistance patterns in nosocomial infections: preliminary data from Hospital of Al-Hoceima, Morocco

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Abstract

Introduction: Nosocomial infections or healthcare-associated infections (HCAIs) represent a public health problem. The burden of antibiotic resistance in these infections is still unclear in Morocco. The objective of this study was to describe antibiotic susceptibility of the main bacteria responsible for nosocomial infections in order to propose prevention measures.

Methodology: Data were collected from 1519 laboratory records including hospital inpatients suspected of nosocomial infections in Mohamed V Hospital of Al-Hoceima between January 2016 and December 2020. The data were analyzed using SPSS software version 25.

Results: Bacteriological test samples included 65.5% of urine, 27.2% of pleural fluid, 4.5% of pus, and 2.8% of protected distal swab. Two hundred and twenty-seven (15%) samples were culture-positive. The bacteria isolated were mainly enterobacteria (*Escherichia coli*, 43.6% and *Klebsiella pneumoniae*, 13%), non-fermentative Gram-negative bacteria (*Pseudomonas aeruginosa*, 10.8%), and *Staphylococcus aureus* (24.3%). Extended spectrum beta-lactamases (ESBLs)-producing Enterobacteriaceae represented 25.4% and those resistant to other families of antibiotics accounted for 12.5%. In our study, we reported 17% ESBL producers among urinary infections. Methicillin-resistant *Staphylococcus aureus* accounted for 22.2%. *Pseudomonas aeruginosa* that were resistant to ticarcillin, ceftazidime, and imipenem represented 29% of the cases.

Conclusions: Our results showed a higher frequency of resistance. A microbiological surveillance system is highly needed to identify bacterial niches in the hospital environment at Mohamed V Hospital.

Key words: resistance; hospital; environment; Morocco; nosocomial infection.

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Introduction

Nosocomial infections or healthcare-associated infections (HCAIs) represent a public health problem due to their significant morbidity and mortality, and their enormous cost [1]. These infections occur in individuals who are under medical care in hospitals or other health care facilities, or even after discharge [2]. Nosocomial infections may be caused by bacteria of endogenous or exogenous origin.

According to the results of two European point prevalence surveys, 4.5 million nosocomial infections were estimated to occur per year in the period 2016 to

2017 in acute care hospitals in the European Union and European Economic Area [3]. These infections were associated with increased mortality (median number of attributable deaths: 33,110) and disability-adjusted life-years (median: 874,541) [4].

A prevalence survey carried out in 27 hospitals in the Eastern Mediterranean Europe region (Algeria, Egypt, Italy, Morocco, and Tunisia) showed a prevalence of nosocomial infections at 10.5% [5]. This was more pronounced in non-university centers and in medium-sized hospitals. However, a meta-analysis concluded that the pooled prevalence of these

nosocomial infections was much higher in developing countries (15.5 per 100 patients) [6,7]. In Morocco, although there are no national legislations that mandate the declaration of all cases of nosocomial infections, the first national survey on nosocomial infections was carried out in 1994 and revealed a prevalence rate of 9%. However, these data were not published [8].

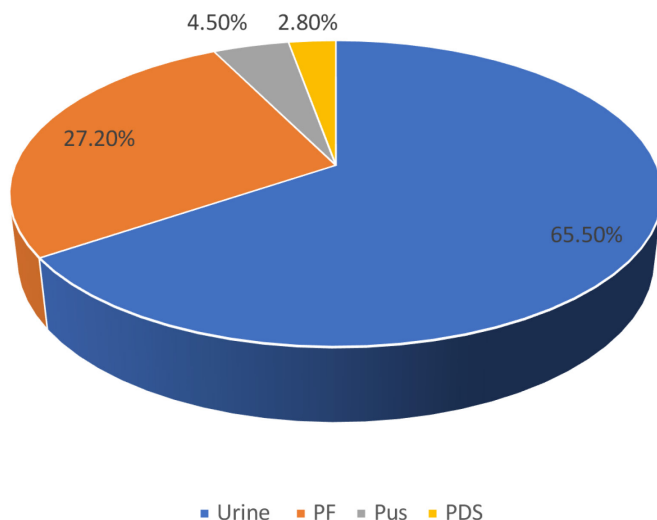
The occurrence of a nosocomial infection has been highly linked to many factors, including invasive procedures, hospitalization more than 8 days, diabetes, and age [9]. Furthermore, exposure to antibiotics is another factor that can cause the emergence of bacterial resistance. This bacterial resistance to antibiotics has become a major public health problem worldwide, with an adverse impact on patient morbidity and mortality [10,11]. In the survey mentioned above, authors reported that 40.7% of patients received antibiotic treatment [5].

The current study is the first of its kind conducted at the Mohammed V Hospital of Al-Hoceima. We aimed to describe the profile and antibiotic resistance of strains that were responsible for nosocomial infections through the analysis of data collected over a period of 5 years.

Methodology

This is a retrospective study conducted in the bacteriology unit of the medical laboratory in the Provincial Hospital Center of Al-Hoceima. The study included the results of microbiological analyses of 1519 clinical samples from hospitalization services during the period between January 2016 and December 2020. Only data of patients who were suspected to have a nosocomial infection were included in this study.

Figure 1. Positivity rate by sample types.



Data were collected from the laboratory records. The specimens were biological fluids, including urine, pleural fluid (PF), pus, and protected distal swab (PDS).

Phenotypical identification and antibiotic susceptibility testing were performed according to the periodically updated recommendations of CASFM-EUCAST (Antibiogram Committee of the French Society of Microbiology-European Committee on Antimicrobial Susceptibility Testing) [12]. In Morocco, the guide for good practices of medical biology analyses is a mandatory quality reference for medical biology laboratories [13]. Bacterial identification of the strains was processed according to the routine methods followed in the laboratory (Gram staining, catalase, DNase, oxidase and API 20E gallery). Strain susceptibility was performed by Muller-Hinton agar diffusion method according to the recommendations of CASFM-EUCAST. Methicillin resistance was detected through susceptibility to 30µg cefoxitin. The extended spectrum beta-lactamases (ESBLs) producing Enterobacteriaceae were determined by testing for synergy between clavulanic acid and third-generation cephalosporins based on the recommendations of CASFM-EUCAST.

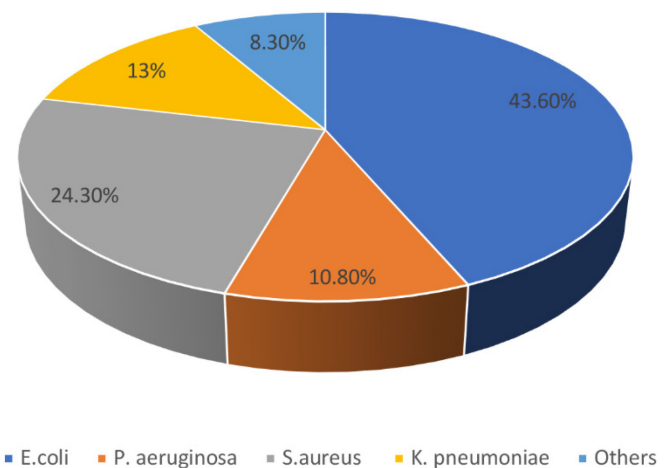
Isolated bacteria were assessed for susceptibility according to the recommendations of CASFM-EUCAST.

Data were analyzed using SPSS software version 25. Correlation analysis were performed by Pearson.

Results

Urine samples were the most frequent (65.5%) out of a total of 1519 bacteriological tests. PF represented 27.2%, pus 4.5%, and PDS accounted for 2.8%. Two hundred and twenty-seven (15%) samples were culture-

Figure 2. Distribution of isolated bacteria.



positive (Figure 1). Isolated bacteria were mainly those that were mostly involved in nosocomial infections, including *E. coli* (43.6%), *S. aureus* (24.3%), *K. pneumoniae* (13%), *P. aeruginosa* (10.8%), and other Gram positive cocci (8.3%) (Figure 2). ESBLs-producing Enterobacteriaceae accounted for 25.4% (32/126).

The most frequently isolated bacterium in urine was *E. coli* with a frequency of 62.4% (83/133). *S. aureus* were mainly isolated in pus with 50%, followed by *K. pneumoniae* in PF (25.6%), and *Pseudomonas aeruginosa* in PDS (21.4%) (Figure 3).

S. aureus and *E. coli* were found in all services, with a predominance in the surgery service (33.3% and 22.6%, respectively). *P. aeruginosa* and *K. pneumoniae*

were mainly identified in the intensive care unit (ICU) and the pneumology service, respectively (66.6% and 34.4%, respectively) (Table 1).

Urine and PUS positive samples were mostly found in the surgery service with 26.8% and 50%, respectively. Whereas the PF positive samples were mainly identified in pneumology service (56.4%) and PDS in the ICU (66.6%) (Table 2).

Regarding antibiotic resistance, *E. coli* in 35% and 24.7% of cases was resistant to ceftazidime and ciprofloxacin, respectively. No other bacteria were resistant to ciprofloxacin. *K. pneumoniae* was resistant to ceftazidime in 44.8% of cases. ESBL-producing Enterobacteriaceae represented 25.4% of cases and those resistant to other families of antibiotics accounted

Table 1. Distribution of isolated bacteria by services.

| Variable | Surgery | Intensive care | Pneumology | Pediatrics | Neonatology | Pediatric surgery | Medicine | Maternity |
|--------------------------------|---------------|----------------|---------------|---------------|--------------|-------------------|---------------|---------------|
| <i>E. coli</i> n = 97 | 22 (22.6%) | 12 (12.3%) | 7 (7.2%) | 14 (14.4%) | 7 (7.2%) | 4 (4.1%) | 16 (16.5%) | 15 (15.4%) |
| <i>P. aeruginosa</i> n = 24 | 0 (0%) | 16 (66.6%) | 0 (0%) | 2 (8.3%) | 0 (0%) | 0 (0%) | 0 (0%) | 6 (25%) |
| <i>S. aureus</i> n = 54 | 18 (33.3%) | 14 (26%) | 2 (3.7%) | 6 (11%) | 3 (5.5%) | 2 (3.7%) | 4 (7.4%) | 5 (9.2%) |
| <i>K. pneumoniae</i> n = 29 | 2 (6.9%) | 4 (13.8%) | 10 (34.4%) | 3 (10.3%) | 3 (10.3%) | 0 (0%) | 5 (17.2%) | 2 (6.9%) |
| Others n = 18 | 2 (11%) | 4 (22%) | 1 (5.5%) | 2 (11%) | 1 (5.5%) | 3 (16.6%) | 2 (11%) | 3 (16.6%) |

E. coli: *Escherichia coli*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. aureus*: *Staphylococcus aureus*; *K. pneumoniae*: *Klebsiella pneumoniae*.

Table 2. Distribution of positive samples by service.

| Variables | Intensive care | Surgery | Pediatrics | Neonatology | Pediatric surgery | Pneumology | Medicine | Maternity |
|-----------|----------------|------------|------------|-------------|-------------------|------------|----------|-----------|
| Urine | 32 (21.4%) | 40 (26.8%) | 14 (9.4%) | 7 (4.7%) | 10 (6.7%) | 4 (2.6%) | 15 (10%) | 27 (18%) |
| Pus | 2 (20%) | 5 (50%) | 0 (0%) | 0 (0%) | 1 (10%) | 0 (0%) | 1 (10%) | 1 (10%) |
| PF | 11 (17.7%) | 4 (6.4%) | 2 (3.2%) | 1 (1.6%) | 3 (4.8%) | 35 (56.4%) | 4 (6.4%) | 2 (3.2%) |
| PDS | 4 (66.6%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 2 (33%) | 0 (0%) | 0 (0%) |

PF: pleural fluid; PDS: protected distal sampling.

Figure 3. Distribution of isolated bacteria by specimens.

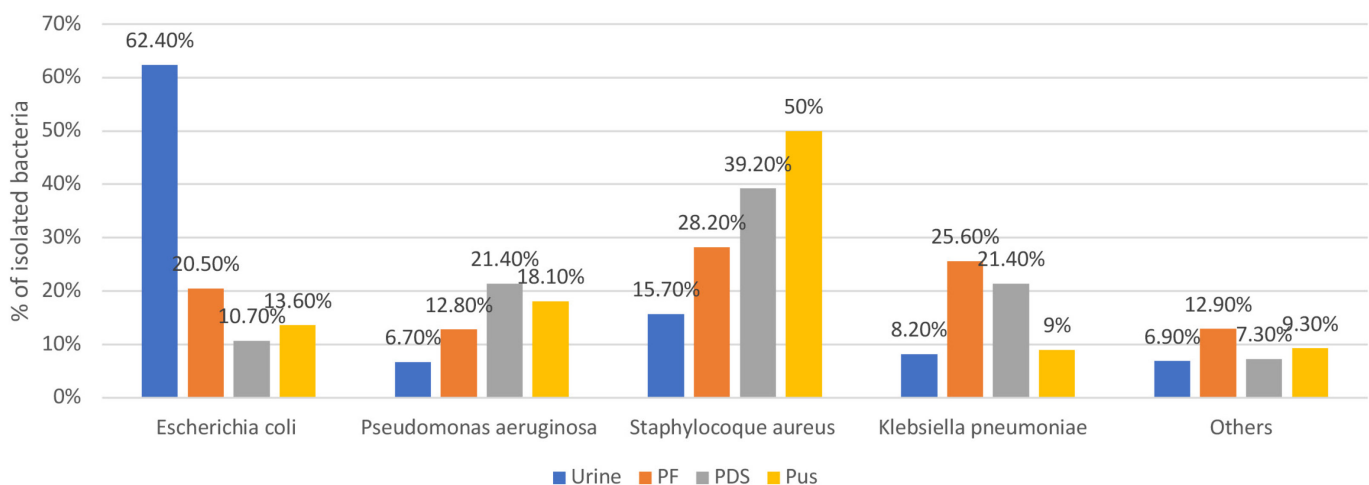


Table 3. Correlation matrix of positive samples.

| Variables | Urine | PUS | PF | PDS |
|-----------|--------------|----------|----------|----------|
| Urine | 1 | | | |
| PUS | 0.823 | 1 | | |
| PF | -0.374 | -0.224 | 1 | |
| PDS | -0.007 | 0.316 | 0.187 | 1 |

PF: pleural fluid; PDS: protected distal sampling; Values are marked in bold when correlation is strong.

for 12.5% of cases. In our study, we reported 17% of ESBL producers among urinary infections. Our results showed that *P. aeruginosa* was resistant to ceftazidime and imipenem with a frequency of 29%, each. No other bacteria were resistant to imipenem. Methicillin-resistant *Staphylococcus aureus* (MRSA) represented 22.2% of cases.

A strong correlation was observed between pus and urine, whereas there was a weak correlation between the PDS and pus samples (Table 3).

We demonstrated a strong correlation between surgery, pediatric, maternity, pediatric surgery, neonatology, and medicine services. A great disparity between these services and ICU and pneumology was described (Table 4).

Discussion

This is the first study reporting data on antibiotic susceptibility of the primary bacteria responsible for nosocomial infections in Mohamed V hospital of Al-Hoceima. Our findings may represent a milestone to establish a monitoring system for these infections in our hospital.

The frequency of nosocomial infections varies between 3.5% and 12% in developed countries. Whereas these infections are more frequent in low resource settings with a rate of up to 19.1% [2]. In Morocco, data about the prevalence of nosocomial infections are limited and the available studies are of low quality. Our frequency was comparable to that reported from a university hospital center (UHC) in Rabat (15% versus 17.8%) [14]. Lower rates were reported in another survey from the same UHC in Rabat (10.3%) [8] and other Moroccan UHCs (Fez with a

frequency of 6.7% and Casablanca with 10.3%) [15,16]. In a systematic review and meta-analysis conducted in 2017 in Morocco which included 11 studies with 4806 patients, the pooled prevalence of nosocomial infections was 9.6% [17].

We demonstrated that *P. aeruginosa* was mostly detected in ICU (66.6%), while *S. aureus* was predominant in the surgery unit (33.3%). Jaffel *et al.* reported that *K. pneumonia* and *S. aureus* were the most frequently isolated bacteria with 26.8 % and 24.1 % in the anesthesia service and ICU of the traumatology and severe burns center in Tunisia [9].

The bacteria isolated from our patients were mostly Gram-negative bacteria, of which *E. coli* represented 29%, and Gram-positive cocci represented 25% of cases, which is in line with other published studies [1,18].

Urinary infections were the most suspected HCAs in our hospital, which explains the higher frequency of cytobacteriological examinations requested by clinicians (65.5%). The rate of urine infection due to Enterobacteriaceae in patients hospitalized in all services was estimated at 62%, those secreting ESBLs represent 15.8% of all Enterobacteriaceae. The greatest number of urinary infections was recorded in the surgical service (31%). *E. coli* was the main microorganism that was frequently isolated in our study (62.4%). This is in accordance with a study in Iran by Nouri *et al.* [19]. In addition, urinary infections were also the most common (9.4%) infections in the pediatric service, with *E. coli* being the most isolated germ (51.8%). Raymond *et al.* reported that 11% of urinary tract infections were in pediatric service, but they showed that *S. aureus* was the most common [20].

Table 4. Correlation matrix between hospitalization services.

| Variables | Intensive care | Surgery | Pediatrics | Neonatology | Pediatric Surgery | Pneumology | Medicine | Maternity |
|-------------------|----------------|--------------|--------------|--------------|-------------------|------------|--------------|-----------|
| Intensive care | 1 | | | | | | | |
| Surgery | 0.405 | 1 | | | | | | |
| Pediatrics | 0.306 | 0.920 | 1 | | | | | |
| Neonatology | 0.139 | 0.914 | 0.980 | 1 | | | | |
| Pediatric surgery | 0.063 | 0.706 | 0.699 | 0.703 | 1 | | | |
| Pneumology | -0.544 | 0.231 | 0.389 | 0.516 | -0.032 | 1 | | |
| Medicine | 0.038 | 0.812 | 0.958 | 0.977 | 0.660 | 0.597 | 1 | |
| Maternity | 0.490 | 0.764 | 0.920 | 0.831 | 0.627 | 0.157 | 0.844 | 1 |

E. coli: *Escherichia coli*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *S. aureus*: *Staphylococcus aureus*; *K. pneumoniae*: *Klebsiella pneumoniae*; Values are marked in bold when correlation is strong.

Another study by Rachel *et al.* reported that *E. coli* was the most prevalent bacteria [21]. Our frequency of urinary infections among women was lower than that reported by Bukasa *et al.* [7], (18% Vs. 37%). However, *E. coli* was also the most identified bacteria (54.5%) in this study, which is in agreement with our results (62.4%).

Our bacteriological profile of PF was dominated by Gram-positive bacteria, of which *S. aureus* represented 28.2%. This is in accordance with Rachidi *et al.* [22] who found that Gram-positive bacteria were dominant (63.2%) from PF, but according to this study *Streptococcus pneumoniae* was the most frequent with 16.4%, followed by *Staphylococcus aureus* with 15.4 %.

E. coli exhibited higher combined resistance to cotrimoxazole and ciprofloxacin. Sbiti *et al.* [23] reported a resistance pattern of ciprofloxacin (92.5%) in ESBLs-producing Enterobacteriaceae. We showed that 29% of *P. aeruginosa* isolated from ICU were resistant to imipenem. These results may be explained by the excessive use of these antibiotics in ICU. Similar antibiotic resistance patterns were described by Lakhdar *et al.* [24] and lvarez-Lerma *et al.* [25].

Several studies described that most nosocomial infections were associated with ESBL-producing bacteria [26]. The prevalence of ESBL-producing Enterobacteriaceae has increased over time across the world. This prevalence varies widely depending on the geographic area. Higher frequency of ESBL-producing Enterobacteriaceae has been reported in Africa, up to 65% [27–29]. A study by Bercion *et al.* showed that the prevalence of uropathogens ESBL-producing Enterobacteriaceae in Bangui, Central African Republic, significantly increased from 3.7% to 19.3% between 2004 and 2006 [28]. In Morocco, the ESBL phenotype rate increased from 3% in 2012 to 11.16% in 2018 [30]. In our study, we reported 25.4% ESBL producers. The ESBL-producing Enterobacteriaceae strains isolated in our hospital were treated by imipenem as they showed susceptibility to carbapenems.

In the present study, a total of 7 carbapenem-resistant Enterobacteriaceae were isolated and identified out of 126 initial Enterobacteriaceae clinical strains (2.4%). This prevalence is three times less than that recorded in a study conducted by Loqman *et al.* during 2018 at Mohamed VI University Hospital, Marrakech, Morocco (8.17%) [31]. However, our frequency remains almost similar to that reported in a study by Wartiti *et al.*, which was conducted over 19 months in a University Hospital in Rabat, Morocco

(2.8%) [32]. We isolated 24.7% ciprofloxacin-resistant *E. coli*, which is comparable to Barguigua *et al.* who reported a frequency of 21.43% [33]. This pattern of low prevalence of carbapenems and ciprofloxacin resistance should be preserved by avoiding inappropriate use of these drugs and controlling the risks of dissemination of ESBLs.

In our study, we identified 22.2% MRSA, which is higher than that described by a study conducted in the University Hospital Center Ibn Rochd of Casablanca reporting a prevalence of 18.4%. This prevalence was higher in the burns (57.7%) and dermatology (39.4%) services. However, this MRSA resistance should be confirmed by molecular tests to determine circulating clones.

We showed that there was a correlation between pus and urine, which means that these samples contained the same bacteria (Table 3). This may be explained by horizontal contamination (via the hands of the nursing staff, insufficiently sterile material or contamination before their use), by the lack of information, lack of training in hospital hygiene, lack of financial resources or because of hospitalization of patients in mixed wards for all specialties. This situation translates to the low level of hospital hygiene and highlights the daily challenge that developing countries are facing in this regard.

We showed a strong correlation between hospitalization services. This may be due to the presence of *E. coli* in all these services. On the other hand, *P. aeruginosa* and *K. pneumoniae* were mostly identified in the ICU and the pneumology unit, respectively, which could explain the discrepancy between these services and others (Table 1 and 4).

Acinetobacter baumannii is considered to be a major cause of nosocomial infections worldwide [34]. We did not find these bacteria in our study. This may be explained by the fact that these data were not noted in the laboratory records. Unfortunately, we did not have strains to repeat microbiological analysis as our study was retrospective and was limited to phenotypic characterization.

Our findings suggested that nosocomial infections acquired during hospitalization and caused by multi-drug-resistant bacteria may impact disease management and lead to failure or even a therapeutic impasse.

In order to maintain patients' safety and improve the quality of care and management, hospitals should adopt global recommendations on hospital hygiene. This requires the establishment of an operational system for the control of nosocomial infections. This system will ensure the presence of a microbiological

surveillance system for the hospital environment, hygiene of the healthcare setting, appropriate management of pharmaceutical and medication waste, and awareness and training of the staff in health care safety and good practices [35,36].

Our study had some limitations. The retrospective character did not allow us to have much information that could be of great interest. Furthermore, we did not carry out the genetic characterization of bacteria as the bacterial strains were not preserved.

Conclusions

Our study showed a predominance of Gram-negative bacilli followed by Gram-positive cocci. Urine was the most infected sample in the surgical service, suggesting that nosocomial infections were most likely of urinary type in our hospital. *E. coli* was the most frequent Gram-negative bacilli isolated in these infections. Moreover, we noted the presence of infections that were caused by *P. aeruginosa* and other ESBL-producing Enterobacteriaceae. In the light of our data, we suggest to avoid horizontal contamination by informing and training staff about nosocomial infections and by separating services. Our preliminary results represent a milestone for further studies to determine the profile of bacteria responsible for nosocomial infections.

Authors' contributions

SB designed the study and drafted the manuscript. FA, AK, and NG participated in the study conception. NM, SB, RB, AS, FA, and EH performed data analysis and interpretation. AM participated in the design of the study, helped with data analysis, writing, interpretation and critical revision of the manuscript. MG helped with statistical analysis and critical revision of the manuscript. All authors have seen and approved the final version of the manuscript.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

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