International Journal of Sciences & Applied Research

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# THE MULTI-DRUG RESISTANT BACTERIA: FREQUENCY AND ANTIBIOTIC RESISTANCE LEVELS IN THE REGION OF MARRAKESH, MOROCCO

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## ABSTRACT

The objective of this study is to establish the frequency of multi-drug resistant bacteria (MDR) isolated at the Avicenne Military Hospital as well as their levels of resistance to antibiotics.

This is a retrospective study conducted over 7years (2015 to 2021), at the Avicenne Military Hospital. Were collected all bacteriological samples received for diagnostic purposes, from patients hospitalized for at least 48 hours. The identification of the bacterial strains was based on the study of their morphological, cultural, and biochemical characteristics on the BD Phoenix™ i100 Automated System, which also allows susceptibility testing. Detection of resistance phenotypes was completed by the conventional method of disc diffusion in an agar medium. The interpretation was based on CA-SFM/EUCAST guidelines.

The overall frequency of MDR bacteria is 14,3% with a large predominance of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae (54,1%) followed by Acinetobacter baumannii resistant to ceftazidime and/or imipenem (28,1%), methicillin-resistant *Staphylococcus aureus* (9,3%), then Enterobacteriaceae resistant to carbapenems (5,2%), and finally *Pseudomonas aeruginosa* resistant to ceftazidime and/or imipenem which represented 3.3% of the MDR bacteria isolated. Co-resistance levels in MDR bacteria are high.

The increasing frequency of multi-drug resistant bacteria highlights the importance of an appropriate antibiotic strategy and the necessity of the implementation of antibiotic resistance monitoring.

**Keywords:** Multi-drug resistant bacteria- Frequency- Antimicrobial resistance- Antibiotics

**Abbreviations:**
MDR: Multi-drug resistant
ESBL: Extended-spectrum β-lactamase
MRSA: Multiresistant Staphylococcus aureus
CPE: Carbapenemase-producing Enterobacteriaceae
MH: Mueller Hinton

## INTRODUCTION

The emergence and spread of multidrug-resistant bacteria (MDR) are major public health issues worldwide. Nosocomial infections caused by MDR bacteria, which are particularly frequent in developing countries, are often associated with high morbidity and mortality and increased costs and hospital stays [1,2]. Knowledge of the local nosocomial bacterial ecology, in particular of the MDR bacteria, and their levels of resistance to antibiotics is an essential step to estimating the extent of the phenomenon, evaluating the effectiveness of control actions, and defining the best adapted therapeutic and prophylactic strategies. The objective of this study is to establish the epidemiological characteristics of the MDR bacteria isolated at the Avicenne Military Hospital of Marrakesh in Morocco as well as their levels of resistance to antibiotics.

## MATERIALS AND METHODS

1- Collection of isolates:

A retrospective study was carried out over a period of 7 years, from 2015 to 2021, at the Avicenne Military Hospital. The investigation covered all bacteriological samples received for diagnostic purposes at the microbiology laboratory. The patients were hospitalized for at least 48 hours. All bacterial strains isolated from samples for ecological purposes and duplicates were therefore excluded.

2- Identification of isolates and susceptibility testing:

The identification of the bacterial strains was based on the study of their morphological, cultural, and biochemical characteristics on the BD Phoenix™ i100 Automated System, which also allows susceptibility testing.

Detection of resistance phenotypes was completed by the conventional agar disc diffusion method. The interpretation was based on CA-SFM/EUCAST guidelines. *(http://www.sfm-microbiologie.org/).*

The study concerned the following MDR bacteria:

-Enterobacteriaceae resistant to 3rd generation cephalosporins by secretion of extended-spectrum beta-lactamase (ESBL),

-Methicillin-resistant *Staphylococcus aureus* (MRSA),

-*Acinetobacter baumannii* resistant to ceftazidime and/or imipenem,

-*Pseudomonas aeruginosa* resistant to ceftazidime and/or imipenem and,

-Enterobacteriaceae resistant to carbapenems.

Strains of Enterobacteriaceae producing an extended-spectrum β-lactamase detected by the automaton were confirmed by a double-disk synergy test between a third-generation cephalosporin and clavulanate. For strains categorized as intermediate sensitivity or resistant to cefotaxime and/or ceftazidime and/or aztreonam, in the absence of synergy between the molecules and clavulanic acid, a cloxacillin test carried out on Mueller Hinton (MH) supplemented with 250 mg/L of cloxacillin (cephalosporinase inhibitor) made it possible to highlight an ESBL possibly masked by a cephalosporinase.

For *S. aureus*, the detection of methicillin resistance was made using a cefoxitin disk (30 µg) under standard antibiogram conditions.

Any Enterobacteriaceae strain with decreased susceptibility to ertapenem (MIC ≥0.5 mg/L or inhibition diameter < 25 mm; 10 µg discs) by agar diffusion test was

considered suspicious for Carbapenemase-producing Enterobacteriaceae (CPE) (CASFM/EUCAST). To improve the detection sensitivity of carbapenemase production, both imipenem and ertapenem were tested.

**RESULTS**

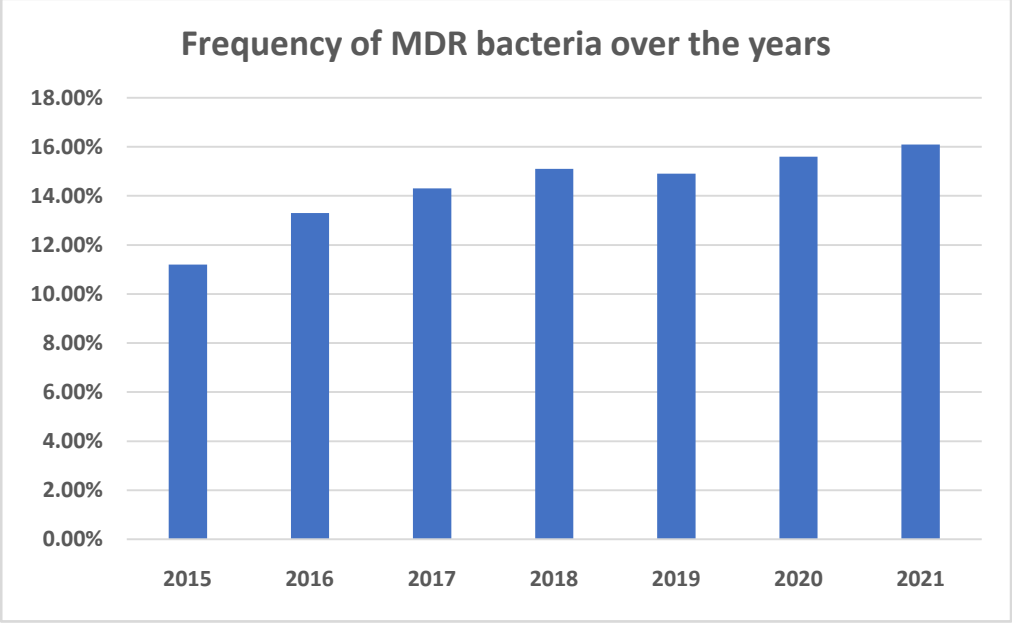
Over 7 years, 615MDR bacteria were collected among 4293 bacterial strains isolated at the Avicenne Military Hospital of Marrakesh. The overall frequency was 14,3%.

The distribution of these MDR bacteria shows a large predominance of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae (54,1%, n=333) followed by *Acinetobacter baumannii* resistant to ceftazidime and/or imipenem (28,1%, n=173), methicillin-resistant *Staphylococcus aureus* (9,3%, n=57), then Enterobacteriaceae resistant to carbapenems (5,2%, n=32), and finally *Pseudomonas aeruginosa* resistant to ceftazidime and/or imipenem (3.3%, n=20). No strains of *Enterococcus faecium* resistant to glycopeptides were isolated.

ESBL-producing strains accounted for 10% of Enterobacteriaceae isolated (total number of Enterobacteriaceae =3486).*K pneumoniae*, and*E. Coli* were most predominant (48% and 32% respectively). Within the *S. aureus* species, MRSA represented14,1% of the isolates (total number of *S. aureus*=405). The percentages of multiresistant *A.baumannii* (total number of *A. baumannii*=216) and multiresistant *Pseudomonas aeruginosa* (total number of *P. aeruginosa*=186) in their species were respectively 80% and 10.7%.

Concerning the evolution of the frequency of MDR bacteria, a gradual increase was noted, especially during the last two years of the study, as shown in figure 1. (Figure 1)

**Figure 1: Frequency of MDR bacteria reported over 7 years**



The departments most affected by these MDR bacteria are respectively intensive care (48%), urology (21%), general surgery (12%), and traumatology (8%).
MDR bacteria were mainly isolated from urinary tract infections (42%), bacteremia (27%), pneumopathy (19%), surgical site infections (11%), and meningitis (1%).

The epidemiological characteristics of patients infected with MDR bacteria showed a male predominance (overall sex ratio of 1.4) with extreme ages ranging from 17 to 87 years. The levels of co-resistance are also important. (Figures 2,3,4).

**Figure 2: Average rates of ESBL Enterobacteriaceae co-resistance to antibiotics**

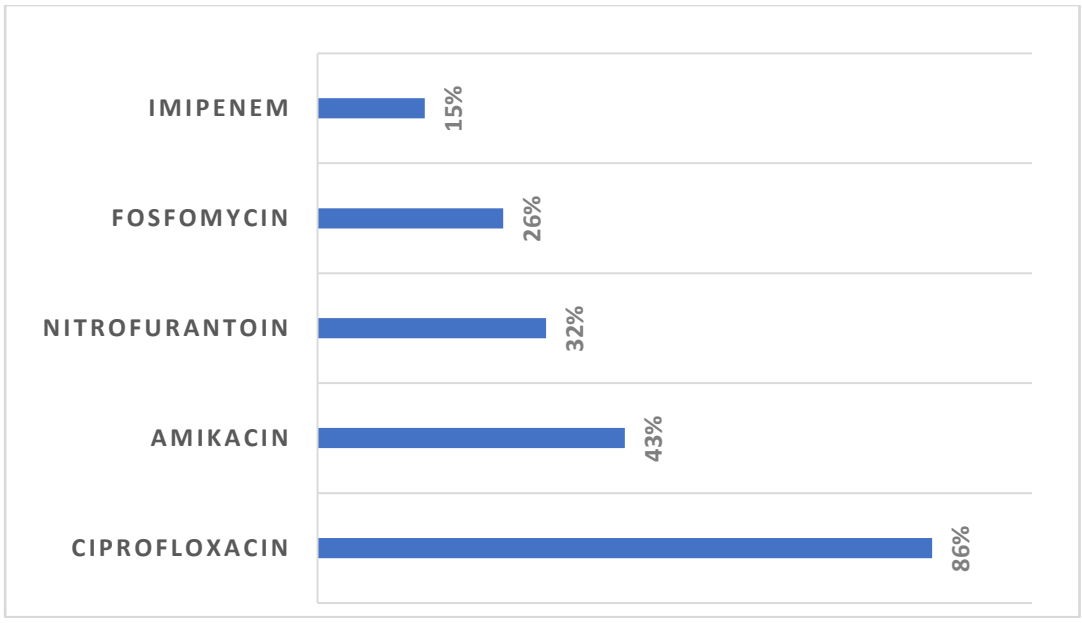



**Figure 3: Average rates of multidrug-resistant A. baumannii co-resistance to antibiotics**

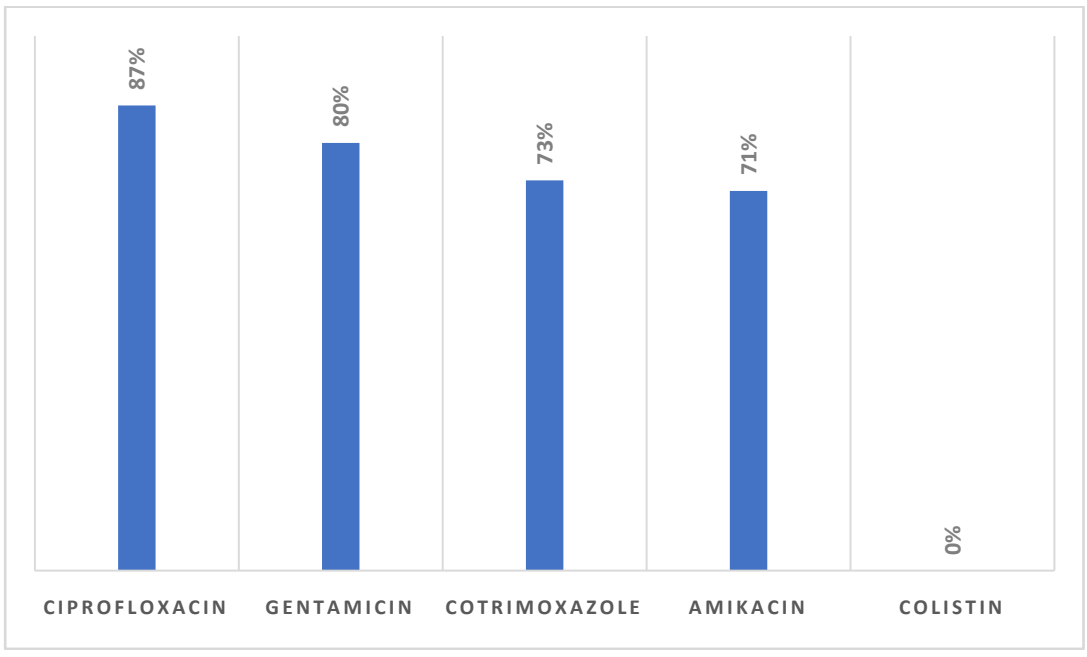
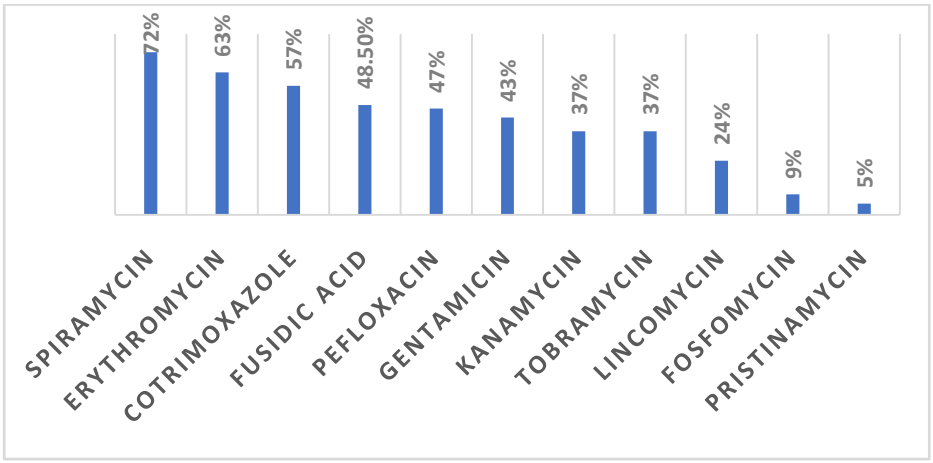


**Figure 4: Average rates of co-resistance to antibiotics in MRSA**




## DISCUSSION

The total frequency of MDR bacteria in this study was 14.3%. This rate is high, and remains higher than that recorded at the University Hospital of Fez, Morocco (11.8%), but below the frequencies reported by another Moroccan University Hospital (33.6% in Rabat, Morocco) and by a Tunisian University Hospital (34.2%) [1-3]. The resurgence of MDR bacteria in hospitals is a worldwide phenomenon but is nevertheless observed in varying proportions depending on the country, and even the region, and more importantly, depending on antibiotic prescription habits and hygiene practices [3].

Among the isolated MDR bacteria, Gram-negative bacilli were the most frequently found in our study, a result consistent with other Moroccan, Tunisian, and French studies [1-4]. This could be explained by the bacterial ecology specific to each hospital.

Our results highlighted a worrying frequency of ESBL-secreting Enterobacteriaceae in our region, although high frequencies (exceeding 30%) have also been recorded in other Moroccan university hospitals, but also in Tunisia and France. [1-5]. The increase in ESBL-secreting Enterobacteriaceae is now observed all over the world not only in nosocomial infections; but in community-acquired infections also [6]. The overuse of broad-spectrum antibiotics has been implicated in the emergence of ESBL-producing strains [7].

According to our study, *K.pneumoniae* is the most isolated among the ESBL producing strains, this agrees with the study by Kooli et al [3] which finds a predominance of Klebsiella, but remains discordant with French and even national data that show a predominance of the species *E .coli* [1,3,4]. Nevertheless, data from various international surveillance networks concerning the prevalence of ESBL-producing organisms in the world show that *K. pneumoniae* is globally predominant in Africa and Europe [5]. The geographical distribution of isolated ESBL is not homogeneous and the prevalence of each species varies considerably from one region to another [6].

The co-resistance rates of the ESBL-producing Enterobacteriaceae isolated are high for ciprofloxacin, gentamicin, amikacin, and sulfamethoxazole-trimethoprim. The rates of co-resistance recorded are close to those reported by a Lebanese study [7].

An essential element in the epidemiology of ESBL is the possibility of transmission of resistance genes between Enterobacteriaceae. Plasmids often carry other resistance genes (in particular to aminoglycosides, tetracyclines, sulfonamides, and trimethoprim) hence the concept of co-resistance, co-expression, and co-selection [8]. As a result, the significant resistance of ESBL Enterobacteriaceae to many families of antibiotics considerably reduces the therapeutic options and maintains a continuous increase in the prescription of carbapenems, particularly of imipenem. According to our study, 1% of the Enterobacteriaceae isolated were resistant to imipenem. The situation relating to Enterobacteriaceae resistant to carbapenems is worrying worldwide [6]. In Morocco, a study carried out by the University Hospital of Rabat reported a prevalence of 2.8% (13/463) [9]. Another study conducted at the University Hospital of Casablanca reported a 6% prevalence of *K. pneumonia* carbapenemase-producing within the species (11/166) [10]. According to Barguigua et al, resistance to imipenem for strains of K. pneumoniae producing ESBL in community settings in Morocco was 14.7% [11]. Some European countries already have high rates like in Romania (14%), even higher in countries like Italy (29%), and approaching 60% in countries like Greece. In France, the resistance of Enterobacteriaceae to carbapenems is currently less of a concern and does not exceed 1% [6].

A. baumannii resistant to beta-lactams represented 28,1% of the MDR bacteria isolated and 80% within the species, with very high rates of co-resistance to antibiotics approaching those noted by the Lebanese study of Harmouche et al [7]. The epidemiological situation of multidrug-resistant A. baumannii infections varies depending on the region, with more or less significant epidemics against a low endemic background in Western Europe, and a rather hyperendemic or permanent epidemic situation in intertropical areas [ 12]. These infections are quite common in developing countries [1,2,7] and represent up to 30 to 40% of mechanically ventilated pneumonia in intensive care units [6]. On the other hand, in France, they are rare and are almost only found in intensive care where they represented only 2% of the microorganisms responsible for nosocomial infections according to the national prevalence survey of 2006 [13]. This increase in the frequency of *A. baumannii* infections is accompanied by an increase in the frequency of resistance to antibiotics for this bacterium [12].

MRSA represented 9,3% of the MDR bacteria isolated and a rate of 14,07% in the species. This rate is close to those found by Moroccan (19.7%) [14], French (22.2% and 22.6%) [15,16], and Tunisian (15.5%) [17] university hospitals. , but remains significantly higher than that reported in the Nordic countries (Sweden, Denmark) where the percentage of *S. aureus* resistant to methicillin has remained very low (< 2%) [14]. The high frequency of MRSA isolation could be mainly due to the significant colonization, essentially nasal, thus facilitating their hand-borne spread in an endemic way in hospitals or even in the community. In addition, the role of environmental reservoirs is highlighted in

the spread of MRSA requiring the implementation of a policy to control the clonal spread of these strains and requires adequate disinfection of surfaces to block the cycle of transmission of MRSA between humans and the environment [15].

Co-resistance rates of MRSA to other antibiotics are high. Methicillin resistance is often associated with other antibiotics such as fluoroquinolones and aminoglycosides; these associated resistances can be explained by the fact that the complex of mec genes determining methicillin resistance is located on a mobile genetic element [14]. The glycopeptides, which constitute the treatment of choice for serious bacteriologically documented MRSA infections, have so far retained excellent activity, according to our study (100% sensitivity), a result consistent with El hamzaoui et al [14]. Different studies have reported the isolation of *S. Aureus*glycopeptides resistant strains, probably due to their misuse in hospitals [17]. Fosfomycin and pristinamycin remain active on more than 90% of our strains, a result comparable to the Tunisian study by Mastouri et al which reported respectively, 7 and 0% resistance to fosfomycin and pristinamycin. Garnier et al, on the other hand, found great resistance to fosfomycin in a pediatric center in the Paris region [18]. In Morocco, fosfomycin retains good activity probably due to its low prescription and its recent commercialization.

In this study, an increase in the frequency of MDR bacteria was noted in the last two years of the investigation (2020-2021). This increase could be explained by the Covid-19 pandemic that lead to a general misuse of antibiotics by the population. In short, the frequencies of MDR bacteria and their resistance levels are very variable depending on time period, country and region.

## CONCLUSION

MDR bacteria constitute a significant infectious risk in our region. Due to their ever-increasing frequency, and the severity of the infections they cause, MDR bacteria represent a great threat in both developed and developing countries. Controlling the spread of these bacteria is based on a rational prescription of antibiotics in order to reduce the selection pressure exerted by broad-spectrum antibiotic therapy, awareness of the entire healthcare team, compliance with standard hygiene precautions, the technical and geographical isolation of patients carrying a transmissible infection or colonized by MDR bacteria and regular surveillance of antibiotic resistance.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest

## AUTHORS CONTRIBUTION

All authors have contributed to the conduct of this work. All authors also declare that they have read and approved the final version of the manuscript.

## ETHICAL CONSIDERATION

All the data has been collected anonymously following patient confidentiality.

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