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# Nosocomial infection caused by multidrug resistant *Enterobacteriaceae* and their spread in inanimate surfaces in East-Algerian hospitals

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**The inanimate hospital environment may serve as a reservoir for resistant bacteria that pose nosocomial infection risks often originating from cross contamination and the most common means of pathogens transference occurs between hands of health professionals, hospital equipment and patients. The aim of this study was to investigate various nosocomial infections with multidrug resistant *Enterobacteriaceae* (MDR-*E*) and their dissemination in hospital surfaces of two Algerian hospitals from January 2014 to December 2014. *Enterobacteriaceae* isolated from hospitalized patients and inanimate surfaces were identified by microbiological methods and confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALD-TOFMS). Antibiotic susceptibility was performed using disk diffusion method. Among 74 nosocomial infections detected, 44 were caused by MDR-*E* (59%) from different clinical specimen; 23 *Klebsiella pneumoniae*, 13 *Escherichia coli*, 5 *Enterobacter cloacae* and 3 *Citrobacter freundii*. From inanimate surfaces, MDR-*E* represents 61% (23 strains out of 38 MDR bacteria isolated); 9 *E. cloacae*, 8 *K. pneumoniae*, 4 *C. freundii* and 2 *E. coli*. In total, 67 MDR-*E* were isolated in 2014. Most *Enterobacteriaceae* show resistance to 13 antibiotics tested out of 15, especially to third-generation cephalosporins, thus resistance to all β-lactams except carbapenems. Here, the dominance of MDR-*E* isolated from nosocomial infections and in hospital surfaces in Algeria and a characterization of *Enterobacteriaceae* strains isolated from different specimens according to their species by MALDI-TOF MS were reported. Interestingly, high level of similarity was found between clinical and environmental strains in antibiotic resistance patterns.**

**Key words:** *Enterobacteriaceae,* multidrug resistance, nosocomial infections, hospital surfaces.

## INTRODUCTION

Nosocomial infections represent a significant health concern. About 60% of some of these infections involve multidrug-resistant (MDR) bacteria (defined as acquired non-susceptibility to at least one agent in three or more

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antimicrobial categories) with an increasing predominance of Gram-negative organisms (Van Duijn et al., 2011; Magiorakos et al., 2012).

Hospitals are now more often facing the problem of antibiotic-resistant nosocomial infections, because of evolution and emergence of bacterial resistance to antibiotics and an increase in the number of immune-suppressed individuals worldwide, drug therapies and genetic disorders (Omenn, 2010).

*Enterobacteriaceae* are inhabitants of the intestinal flora and are among the most frequently isolated strains from hospitalized patients. They are the source of nosocomial infections. They have the propensity to spread easily between humans (hand carriage, contaminated food and water), causing infections such as cystitis and pyelonephritis with fever, septicemia, pneumonia, peritonitis, meningitis and device-associated infections (Nordmann et al., 2011; Paterson, 2006).

The emergence and spread of resistance in *Enterobacteriaceae* are complicating the treatment of serious nosocomial infections (Paterson, 2006). Current antimicrobial resistance profile of *Enterobacteriaceae* include the spread of non-susceptible strains to third-generation cephalosporins, recently, the emergence of carbapenem-resistant *Enterobacteriaceae* has been reported (Nordmann et al., 2011).

*Enterobacteriaceae* are supposed to be cross-transmitted like other nosocomial pathogens with the transient skin flora after direct contact with a colonized patient in the absence of hand hygiene. However, contamination of hands or clothes of healthcare workers could occur not only following direct contact with patients but also with the environment (Touati et al., 2008). Numerous nosocomial outbreaks of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* have been reported worldwide (Touati et al., 2008). Also, their dissemination in hospital surfaces was reported (Jalapoor, 2011).

Previous study have detected extended spectrum of β-lactamases (ESBL) producing *Enterobacteriaceae* in environmental and clinical specimens in a surgery intensive care unit (Kac et al., 2004). In Algeria, several studies have shown the spread of multidrug-resistant *Enterobacteriaceae* (MDR-E) in hospitals (Baba Ahmad-Kazi et al., 2014). Recently, the presence of ESBLs enzymes in *Enterobacteriaceae* clinical and environmental isolates in Algerian hospitals was reported (Touati et al., 2008, 2010).

The present study was designed to investigate nosocomial infection with MDR-*E* and their spread in various inanimate areas of two East-Algerian hospitals in the same period.

## MATERIALS AND METHODS

### Sampling

The study was carried out from 01 January to 31 December 2014 in 2 hospitals in Guelma, East of Algeria. Nine wards were included in this study: emergency, operating room, pediatric, gynecology and neonatal, general surgery and general medicine in the hospital A; infectious diseases, phthisiology and hemodialysis in hospital B.

The present study was carried out on MDR-*E* strains isolated from patients hospitalized for at least 48 h, and from environmental samples (inanimate surfaces and adjacent equipment). Clinical strains included in this study were isolated from urine, pus, tracheal aspiration and blood, according to the diagnosis.

Environmental swabs samples were carried in inanimate area of approximately 10 cm² (patients beds, door's handles, patient's tables, treatment trolley, tap water, infusion stand, stretcher, siphon, radiator, treatment bench-top, drugs tray and nurse's hands) by means of a sterile swab moistened with nutrient broth medium (Algeria Pasteur institute) (Sehulster et al., 2003). After sampling, swabs were incubated in the nutrient broth medium at 37°C for 24 h, then; the strains were subcultured and isolated on Mac Conkey agar (Bio Rad).

### Bacterial strains

The identification of bacteria was performed with microbiological methods (Gram stains, oxydase test and API20E identification system « bio-Mérieux ») (www.biomerieux.fr) and confirmed by matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF MS) method (Microflex; Bruker Daltonics) using 96 spot polished-steel targets (Seng et al., 2009). The peak profiles of identified strains were compared and analyzed using Biotyper 3.0 software (Bruker Daltonics) to build a dendrogram of mass spectral data (Bakour et al., 2012).

### Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed according to the antibiotic susceptibility standard disc diffusion method on Mueller-Hinton agar as recommended by EUCAST (2013) (www.sfm-microbiologie.org/). The susceptibility of the isolates was determined using discs of amoxicillin (25 µg), amoxicillin-clavulanate (20/10 µg), ticarcillin–clavulanate (75/10 µg), céfoxitine (30 µg), aztreonam (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), imipenem (10 µg), amikacine (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), trimithoprime-sulfamethoxazol (1.25/23.75 µg), rifampicin (30 µg), fosfomycine (50 µg) and colistin (50 µg) (Bio-Rad). The results were interpreted according to the recommendations of EUCAST (2013).

## RESULTS

### Bacterial isolates

Over the period of this study (January 2014 to December 2014), 112 multidrug-resistant bacteria were isolated, of which, 74 were isolated from different clinical specimen of nosocomial infections, and 38 were surfaces isolates. Sixty-seven of them (67/112) were MDR-*E*, where, 44 out of 74 (59%) were isolated from clinical samples: 23 *Klebsiella pneumoniae*, 13 *Escherichia coli*, 5 *Enterobacter cloacae* and 3 *Citrobacter freundii*. However, as for inanimate surfaces, 23 (61%) out of 38 MDR-*E* were identified: 9 *E. cloacae*, 8 *K. pneumoniae*, 4 *C. freundii* and 2 *E. coli*.

Majority of the strains were recovered from infectious

**Table 1.** The distribution of multidrug resistant *Enterobacteriaceae* isolated from clinical and surfaces samples according to the hospitals wards.

| Wards | Clinical isolates n (%) | Surface isolates n (%) |
|---|---|---|
| Emergency[A] | 0 (0) | 3 (13) |
| Operating room[A] | 0 (0) | 0 (0) |
| Pediatric[A] | 10 (23) | 3 (13) |
| Gynecology and neonatal[A] | 8 (18) | 0 (0) |
| General surgery[A] | 6 (14) | 5 (22) |
| General medicine[A] | 1 (2) | 0 (0) |
| Infectious diseases[B] | 13 (30) | 6 (26) |
| Phthisiology[B] | 5 (11) | 5 (22) |
| Hemodialysis[B] | 1 (2) | 1 (4) |
| Total | 44 (100%) | 23 (100%) |

n. Number; A. hospital A; B. hospital B.

**Table 2.** The distribution of *Enterobacteriaceae* isolates according to the clinical samples type.

| Sample type | Isolates n (%) |
|---|---|
| Urine | 38 (86) |
| Pus | 3 (7) |
| Tracheal aspiration | 2 (5) |
| Blood | 1 (2) |
| Total | 44 (100%) |

**Table 3.** The distribution of multidrug resistant *Enterobacteriaceae* isolated from inanimate surfaces.

| Surface | Isolates n (%) |
|---|---|
| Door's handles | 5 (22) |
| Siphons | 4 (18) |
| Taps water | 2 (9) |
| Stretchers | 2 (9) |
| Patient's beds | 2 (9) |
| Patient's tables | 2 (9) |
| Nurse's hands | 1 (4) |
| Treatment trolley | 1 (4) |
| Radiator | 1 (4) |
| Treatment bench-top | 1 (4) |
| Infusion stand | 1 (4) |
| Drugs tray | 1 (4) |
| Total | 23 (100%) |

diseases ward (19 MDR-*E* out of 67), of them, 13 (30%) were from patient samples, and 6 (26%) from inanimate surfaces (Table 1). On the other hand, urinary nosocomial infection was the predominant, urine was the source of 86% of MDR-*E* (38 strains) (Table 2). However, source of environmental samples have a close rate, of which, door's handles was the predominant with 22% (Table 3).

An MSP dendrogram was constructed using MALDI-TOF spectra of MDR-E isolates through Biotyper 3.0 software (Bruker Daltonics, Bremen, Germany). Four groups were found to correspond to a 4 cluster obtained at the arbitrary distance value of 100. Each cluster corresponds to a species, by grouping the clinical strains and those of the surfaces belonging to the same species (Figure 1).

## Antimicrobial susceptibility test

The results of *in vitro* susceptibilities to 15 antimicrobial agents test for clinical and surfaces isolates of 4 common species of *Enterobacteriaceae*, revealed high-level resistance to antibiotics, especially to β-lactams, except imipenem, with 100% of resistance to amoxicillin/clavulanic acid, ticarcillin/clavulanic acid, cefotaxime and ceftriaxone. The percentage of cefoxitine range from 41% for clinical strains to 65% for surfaces, and aztreonam, range from 82% for clinical to 92% for surfaces isolates.

From high to moderate resistance to trimethoprim-sulfamethoxazole, aminoglycosides, fluoroquinolones and fosfomycin were detected: 64 and 78% for trimethoprim-sulfamethoxazole from clinical and surfaces strains, respectively, and the rate of resistance to gentamicin ranging from 64 to 74% and 23 to 0% for amikacine, 48 to 44% for ciprofloxacin, and that of fosfomycine range from 52 to 44% for clinical and surfaces strains, respectively. A high level resistance to rifampicin was detected, 91% for both, clinical and surfaces strains. No resistance was detected for colistin (Figure 2). Interestingly, 76% of clinical and surfaces strains presented 100% of similarity in the antibiotic resistance patterns.

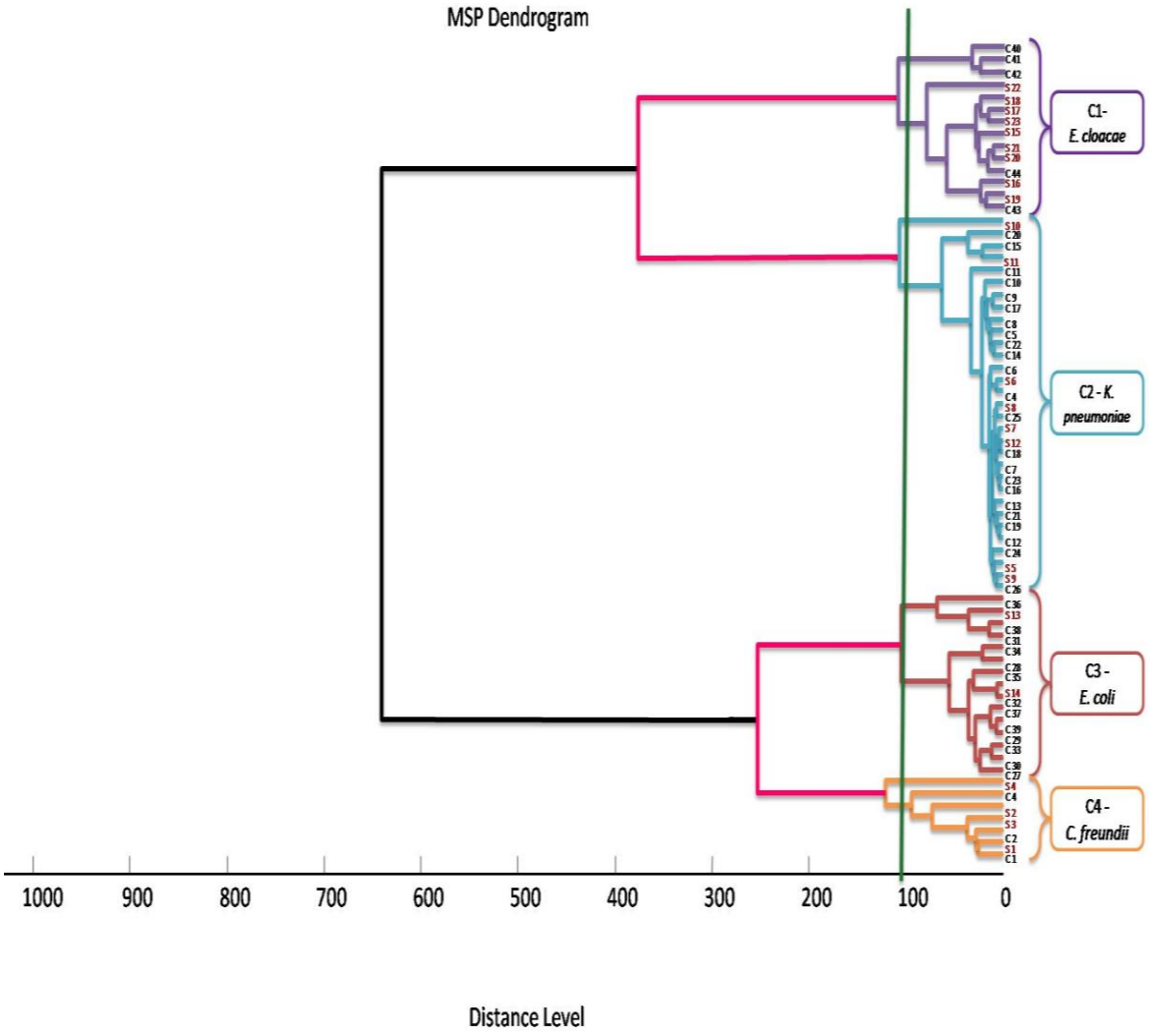


**Figure 1.** Cross-wise minimum spanning dendrogram generated by the Biotyper 2.0 program for the 67 MDR-*Enterobacteriaceae* clinical and surfaces strains isolated from East-Algerian hospitals (A and B). The arbitrary distance level at 100 is indicated as vertical green lines. Clustering of the strains according to this cut-off value significantly associates all *E. cloacae* strains in one cluster C1, *K. pneumoniae* in C2, *E. coli* in C3 and associates all *C. freundii* strains in C4. (Strains code: Black: clinical strains, Red surfaces strains).

## DISCUSSION

Bacterial contamination of touch surfaces pose a potential risk of nosocomial infection, in Algeria. ESBL-producing *Enterobacteriaceae* in hospital surfaces and the clonal relatedness between clinical and surfaces isolates where previously reported (Touati et al., 2008, 2010). This study shows that multidrug resistant *Enterobacteriaceae* continue to be associated with hospital-acquired infections in Algerian hospitals.

Several studies reported the prevalence of *Enterobacteriaceae* as causative of nosocomial infections, in India; a recent study revealed that *Enterobacteriaceae* are the most frequently isolated in hospitals (Sahu et al., 2016), this concur with the high frequency of *Enterobacteriaceae* (59%) revealed in the present study. In the present study, the proportion of surfaces contamination with MDR-*E* was 61%. This proportion is higher than the rates revealed in previous studies (9, 10.7 and 26%) (Touati et al., 2010; Kac et al., 2004; Jalapoor, 2011).

To the authors' knowledge, several studies demonstrate the rate of nosocomial infections and the contamination of hospital environment in intensive care units, pediatric and neonatal wards. In this study, the ward most affected by nosocomial infections with contamination of their surfaces was infectious disease ward.

This study has revealed that urinary nosocomial infection was the most detected; several studies have

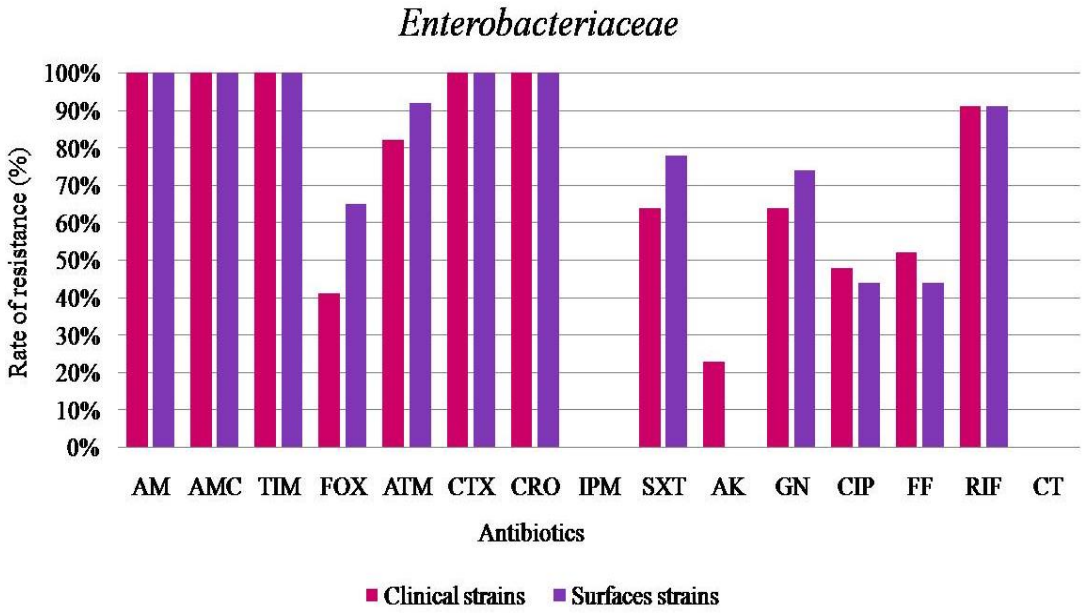


**Figure 2.** Antimicrobial resistance pattern among clinical and surfaces strains. AM. Amoxicillin, AMC. amoxicillin-clavulanate, TIM. ticarcillin–clavulanate, FOX. cefoxitine, ATM. aztreonam, CTX. cefotaxime, CRO. ceftriaxone, IMP. imipenem, SXT. trimithoprime-sulfamethoxazol, AK. amikacin, GN. gentamicin, CIP. ciprofloxacin, FF. fosfomycine, RIF. rifampicin, and CT. colistin.

shown 4 most frequent types of hospital-acquired infection: pneumonia, surgical site infection, urinary tract infection and bloodstream infection (Gaynes et al., 2005). Literatures have previously reported that door handles were the main source of bacteria and this is consistent with this study (De Abreu et al., 2014; Taneja et al., 2004). MALDI-TOF MS is mostly used as the main tool for species identification (Seng et al., 2010; Berrazeg et al., 2013). It is a rapid and alternative method for a better epidemiology survey of these bacteria, especially for a suspected outbreak and/or emergence of specific clones, in order to implement rapid infection control measures (Bakour et al., 2012)

Currently, some studies are focused on whether is possible to use MALDI-TOF MS as a discriminatory tool for typing (Mesli et al., 2013; Batah et al., 2015; Novais et al., 2014; Sachse et al., 2014).

The analysis of the dendrogram generated by Biotyper software showed that MALDI-TOF MS was useful to characterize the *Enterobacteriaceae* strains according to their species; the protein signatures formed 4 separate clusters related to each one the species. This is consistent with the recent findings where authors showed that MALDI-TOF MS is able to identify and class *Acinetobacter* species strains in separate clusters (Mesli et al., 2013). These results propose that the isolated strains of the same species either of clinical or environmental origin, are very close in their protein structure, it suppose that the grouping of species isolated from different wards of two hospitals could be due to either patient-to-patient, visitor to patients transmission or to the contamination of nurse's hands and hospital surfaces and equipments.

In these series of strains, all *Enterobacteriaceae* presented high level resistance to most antibiotics tested, this results have been likely previously reported in several studies worldwide, either clinical or surfaces isolates (Karlowsky et al., 2003; Touati et al., 2010; Sahu et al., 2016; Jalapoor, 2011).

Another important finding in this study is that most environmental *Enterobacteriaceae* isolates have similar MDR profiles of strains isolated from hospitalized patients over the same period. All recent studies from different countries have shown increasing incidence of MDR clinical and surfaces isolates of *Enterobacteriaceae* (Kac et al., 2004; Touati et al., 2008).

Finally, contamination of hospital surfaces with multidrug resistant *Enterobacteriaceae* is a potential source for nosocomial infections which can be transmitted from patient to patient, hospital surfaces and equipments to patients, also from hospital staff to patients. Importantly, several articles demonstrate that enhanced cleaning, the use of no-touch methods for terminal room disinfection, and potentially the use of self-disinfecting surfaces may help in reducing nosocomial infections and contamination of hospital environment.

## Conflict of interest

All the authors have declared that there is no conflict of interest.

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### REFERENCES

Baba Ahmed-Kazi Tani Z, Arlet G (2014). Actualité de la résistance aux antibiotiques chez les bacilles à Gram négatif en Algérie. J. Pathol. Biol. 62:169-178.

Bakour S, Kempf M, Touati A, Ait Ameur A, Haouchine D, Sahli F, Rolain JM (2012). Carbapenemase-producing *Acinetobacter baumannii* in two university hospitals in Algeria. J. Med. Microbiol. 61:1341-1343.

Batah R, Loucif L, Olaitan AO, Boutefnouchet N, Allag H, Rolain JM (2015). Outbreak of *Serratia marcescens* Coproducing ArmA and CTX-M-15 Mediated High Levels of Resistance to Aminoglycoside and Extended-Spectrum Beta-Lactamases, Algeria. Microb. Drug Resist. 21:470-476.

Berrazeg M, Diene SM, Drissi M, Kempf M, Richet H, Landraud L, Rolain JM (2013). Biotyping of multidrug-resistant *Klebsiella pneumoniae* clinical isolates from France and Algeria using MALDI-TOF MS. PLoS One 8:e614-e628.

De Abreu PM, Farias PG, Paiva GS, Almeida AM, Morais PV (2014). Persistence of microbial communities including *Pseudomonas aeruginosa* in a hospital environment: a potential health hazard. BMC Microbiol. 14:118.

EUCAST-European Committee on Antimicrobial Susceptibility Testing (2013). Breakpoint tables for interpretation of MICs and zone diameters. Version 1.0. EUCAST.

Gaynes R, Edwards JR (2005). Overview of nosocomial infections caused by gram-negative bacilli. Clin. Infect. Dis. 41:848-854.

Jalapoor S (2011). Study of the antibiotic resistance pattern among the bacterial isolated from the hospital environment of Azzahra Hospital, Isfahan, Iran. Afr. J. Microbiol. Res. 5:3317-3320.

Kac G, Podglajen I, Vaupre S, Colardelle N, Buu-Hoi A, Gutmann L (2004). Molecular epidemiology of extended-spectrum β-lactamase-producing *Enterobacteriaceae* isolated from environmental and clinical specimens in a cardiac surgery intensive care unit. J. Hosp. Infect. 25:e852-e855.

Karlowsky JA, Jones ME, Thornsberry C, Friedland IR, Sahm DF (2003). Trends in antimicrobial susceptibilities among *Enterobacteriaceae* isolated from hospitalized patients in the United States from 1998 to 2001. Antimicrob. Agents Chemother. 47:1672-1680.

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 18:268-281.

Mesli E, Berrazeg M, Drissi M, Bekkhoucha SN, Rolain JM (2013). Prevalence of carbapenemase-encoding genes including New Delhi metallo-beta-lactamase in *Acinetobacter* species, Algeria. Int. J. Infect. Dis. 17:e739-e743.

Nordmann P, Naas T, Poirel L (2011). Global Spread of Carbapenemase producing *Enterobacteriaceae*. Emerg. Infect. Dis. 18:1791-1798.

Novais A, Sousa C, deDios CJ, Fernandez-Olmos A, Lopes J, Ramos H, Coque TM, Canton R, Peixe L (2014). MALDI-TOF mass spectrometry as a tool for the discrimination of high-risk *Escherichia coli* clones from phylogenetic groups B2 (ST131) and D (ST69, ST405, ST393). Eur. J. Clin. Microbiol. Infect. Dis. 33:1391-1399.

Omenn GS (2010). Evolution and public health. Proc. Natl. Acad. Sci. U.S.A. 107:1702-1709.

Paterson DL (2006). Resistance in gram-negative bacteria: *Enterobacteriaceae*. Am. J. Infect. Control 34:S20-S28.

Sachse S, Bresan S, Erhard M, Edel B, Pfister W, Saupe A, Rodel J (2014). Comparison of multi locus sequence typing, RAPD, and MALDI-TOF mass spectrometry for typing of beta-lactam-resistant *Klebsiella pneumoniae* strains. Diagn. Microbiol. Infect. Dis. 80:267-271.

Sahu MK, Siddharth B, Choudhury A, Vishnubhatla S, Singh SP, Menon R, Kapoor PM, Talwar S, Choudhary S, Airan B (2016). Incidence, microbiological profile of nosocomial infections, and their antibiotic resistance patterns in a high volume Cardiac Surgical Intensive Care Unit. Ann. Card. Anaesth. 19:281-287.

Sehulster L, Chinn RY (2003). Guidelines for environmental infection control in health-care facilities. Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC). MMWR Recomm. Rep. 52:1-42.

Seng P, Drancourt M, Gouriet F, La Scola B, Fournier PE, Rolain JM, Raoult D (2009). Ongoing revolution in bacteriology: routine identification of bacteria by matrix assisted laser desorption ionization time-of flight mass spectrometry. Clin. Infect. Dis. 49:543-551.

Seng P, Rolain JM, Fournier PE, La Scola B, Drancourt M, Raoult D (2010). MALDITOF-mass spectrometry applications in clinical microbiology. Future Microbiol. 5:1733-1754.

Taneja N, Emmanuel R, Chari PS, Sharma M (2004). A prospective study of hospital-acquired infections in burn patients at a tertiary care referral centre in North India. Burns 30:665-669.

Touati A, Brasme L, Benallaoua S, Madoux J, Gharout A, De Champs C (2008). *Enterobacter cloacae* and *Klebsiella pneumoniae* isolates producing CTX-M-15 recovered from hospital environmental surfaces from Algeria. J. Hosp. Infect. 68:183-185.

Touati A, Zenati K, Brasme L, Benallaoua S, De Champs C (2010). Extended-spectrum beta-lactamase characterisation and heavy metal resistance of *Enterobacteriaceae* strains isolated from hospital environmental surfaces. J. Hosp. Infect. 75:78-79.

Van Duijn PJ, Dautzenberg MJ, Oostdijk EA (2011). Recent trends in antibiotic resistance in European ICUs. Curr. Opin. Crit. Care 17:658-665.