

Multi-resistance, extended and pan-resistance to antimicrobials at the North of México

Multirresistencia, resistencia extendida y panresistencia a antibacterianos en el norte de México

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Abstract

Objective: To describe the profile of multidrug-resistance (MDR), extensively resistance (XDR) and pandrug-resistance (PDR) to antibacterial drugs in isolates from patient samples from a third level private hospital in the North of Mexico. **Method:** A retrospective study was carried out during the period from February 2016 to April 2019. From 156 clinical samples of urine, wounds, blood, expectoration and other fluids, 166 bacteria were isolated; 10 samples included two microorganisms. Isolated microorganisms were classified into MDR, XDR or PDR. **Results:** 78% of the Gram negative and 69% of the Gram positive isolates showed MDR, XDR or PDR. *Staphylococcus epidermidis* was the most frequently isolated MDR Gram positive bacteria. *Escherichia coli* and *Klebsiella* sp. were among the most frequent MDR Gram negative. In two cases, the clinical isolates of *Pseudomonas aeruginosa* from the neonatal intensive care unit showed PDR. **Conclusions:** The intensive care, surgery and neonatal intensive care unit services deserve special attention due to the high proportion of MDR isolates and the presence of PDR due to *P. aeruginosa*.

Keywords: Bacteria. Multidrug resistance. Extended resistance. Pan-resistance. Mexico.

Resumen

Objetivo: Describir el perfil de multirresistencia (MDR), resistencia extendida (XDR) y panresistencia (PDR) a antibacterianos en aislados de muestras de pacientes de un hospital privado de tercer nivel en el norte de México. **Método:** Se realizó un estudio retrospectivo durante el periodo comprendido de febrero de 2016 a abril de 2019. A partir de 156 muestras clínicas de orina, heridas, sangre, expectoración y otros fluidos se aislaron 166 bacterias; 10 de las muestras incluyeron dos microorganismos. Los microorganismos aislados se clasificaron en MDR, XDR o PDR. **Resultados:** El 78% de los aislados gram-negativos y el 69% de los aislados grampositivos mostraron MDR, XDR o PDR. *Staphylococcus epidermidis* fue la bacteria grampositiva con multirresistencia más frecuentemente aislada. *Escherichia coli* y *Klebsiella* sp. se encontraron entre los gramnegativos MDR más frecuentes. En dos casos, los aislados clínicos de *Pseudomonas aeruginosa* procedentes de la

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unidad de cuidados intensivos neonatales mostraron PDR. **Conclusión:** Los servicios de terapia intensiva, cirugía y unidad de cuidados intensivos neonatales merecen especial atención por la alta proporción de aislados MDR y la presencia de PDR a causa de *P. aeruginosa*.

Palabras clave: Bacteria. Multirresistencia. Resistencia extendida. Panresistencia. México.

Introduction

Antimicrobial resistance refers to the processes presented by microorganisms (bacteria, viruses, fungi and parasites) that affect and make for the drugs used in their treatment to be inefficient¹.

The shortage, and in some cases absence, of efficacious drugs for the treatment of infectious diseases has serious consequences. Data from the World Health Organization (WHO) indicate that bacterial resistance caused 700,000 deaths in 2015; this figure, according to the study carried out by O'Neill², could rise to 10 million by 2050 if interventions are not implemented to revert the trend. According to WHO recent projections, in the next 30 years, deaths caused by resistant bacteria could be more common than those attributed to cancer^{3,4}. In economic terminology, resistance is a negative externality, i.e., it has an undesirable effect on other people in addition to immediate consequences on the antibiotic consumer⁵.

An increasing number of infections, such as pneumonia, tuberculosis, septicemia and gonorrhea, are becoming more difficult and sometimes impossible to treat, as antimicrobials lose efficacy^{1,6}. Never has the threat of antimicrobial resistance been more immediate and the need for solutions more urgent, says Dr. Tedros Adhanom Ghebreyesus, WHO Director-General.

Although reports on antibacterial agents under development are groundbreaking, it will be years before they reach patients, and new treatments by themselves will not be enough to combat the threat of antimicrobial resistance; for this reason, the WHO works with different countries to improve infection prevention and control, and to promote appropriate use of existing and future antibiotics^{7,8}.

Antimicrobial resistance can be natural (intrinsic) or acquired. Natural resistance is the result of specific properties of the microorganism and it appears prior to the use of antibiotics. Acquired resistance is defined as a loss of sensitivity to the therapeutic concentrations used of a particular drug. Strictly speaking, a microorganism with in vitro-acquired resistance to more than one antibacterial drug is considered to be multidrug-resistant (MDR), but this multidrug

resistance can be stratified into levels. International definitions of MDR, extensively drug-resistant (XDR) and pan-drug-resistant (PDR) were proposed in 2012⁹. MDR per se is regarded as that of a microorganism that is not sensitive to at least one antimicrobial agent of three or more pharmacological families. XDR is presented by a microorganism that is not sensitive to at least one agent in all but one or two antimicrobial categories. PDR refers to a microorganism that is resistant to all drugs of all categories. Proper use of these definitions allows a better understanding of the extent of the resistance problem and enables the comparison of epidemiological surveillance data between institutions, regions and countries⁹. For being able to design and implement targeted interventions whose outcomes can be measured, it is essential for information on antimicrobial resistance to be organized into a specific knowledge of the characteristics and magnitude of the problem. The present study has the purpose to characterize the bacterial resistance profile in a private tertiary care hospital.

Method

An observational, cross-sectional, retrospective study was carried out during the period from February 2016 to April 2019 in a private tertiary care hospital, located in the city of Chihuahua, Chihuahua, Mexico. The sample size was calculated according to the number of existing cultured clinical samples during the above-mentioned period. A total of 166 bacteria were isolated (Table 1). Identification of microorganisms was carried out from different cultures, and their sensitivity to antibiotics was determined with the BD Phoenix automated system, complying with the internal policies of the institution's laboratory and the Clinical and Laboratory Standards Institute (CLSI) standards¹⁰. Isolated microorganisms were classified as MDR, XDR or PDR according to the definitions of the European Center for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) of the United States of America⁹⁻¹¹. Additional information was extracted from medical records, such as the department in charge of patient

Table 1. Type of clinical samples, number of bacterial isolates and level of resistance

Clinical sample	No. of samples	No. of isolates	Level of resistance			
			MDR	XDR	PDR	Non-MDR
Urine	23	25	16	0	0	9
Wound/eschar exudate	21	24	17	1	0	6
Blood culture/catheter	48	48	31	4	1	12
Bronchial expectoration/secretion	48	53	40	2	1	10
Other	16	16	10	0	0	6
Total	156	166	114	7	2	43

MDR: multi-resistance; PDR: pan-resistance; XDR: extended-resistance.

admission. The variables of interest were recorded in a database.

The study was approved by the Research Committee of the Faculty of Medicine and Biomedical Sciences (CI-057-19), and patient personal data were not used. Descriptive statistical tests were used. Percentage values were obtained by bacteria species and hospital area.

Results

A total of 156 clinical samples from various biological fluids were cultured: urine (n = 23), wound or eschar exudate (n = 21), blood and catheter tip (n = 48), bronchial expectoration and secretion (n = 48), and others (e.g., cerebrospinal, peritoneal, and pulmonary fluid; n = 16). From these samples, 166 bacterial strains were characterized because, in 10 of the analyzed clinical samples, two bacteria were isolated (Table 1).

In total, 58% (96/166) of bacteria were gram-negative, with *Escherichia coli* (29/166), *Pseudomonas aeruginosa* (18/166) and *Klebsiella pneumoniae* (14/166) being found to be among the most common; the most common gram-positive bacteria (70/166) included: *Staphylococcus epidermidis* (22/166), *Enterococcus faecalis* (14/166) and *Staphylococcus aureus* (10/166) (Table 2).

The antibiotic sensitivity profile was used to classify bacteria as non-MDR (when they showed no multidrug resistance), MDR, XDR or PDR. Figure 1 shows that 75 gram-negative bacteria (78%) had some level of multidrug resistance: 66 were found to be MDR, seven were XDR and, notoriously, two were found to be PDR. On the other hand, 48 (69%) gram-positive bacteria isolates were MDR. A characterization by species and their MDR level are shown in table 3.

Table 2. Classification of isolated bacteria according to Gram stain and most common species

Classification	n (%)	Main representatives (in descending order)
Gram-negative	96 (58)	<i>E. coli</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i>
Gram-positive	70 (42)	<i>S. epidermidis</i> , <i>E. faecalis</i> , <i>S. aureus</i>
Total	166 (100)	

The 166 bacteria belonged to one of 33 different species; only three of the 33 isolated species did not show any MDR level (*Pseudomonas oryzihabitans*, *Citrobacter freundii* and *Staphylococcus schleiferi*). *P. aeruginosa* showed all three levels of multiple resistance (MDR: eight isolates; XDR: three isolates; PDR: two isolates). At least one *K. pneumoniae*, *Klebsiella aerogenes*, *Acinetobacter baumannii* and *Burkholderia cepacia* complex isolate was MDR and XDR. Finally, 25 species showed at least one isolate with MDR. This shows that multidrug resistance in the sample covers all levels and almost all included species.

As for the type of biological sample and resistance level (Table 1), all urine isolates that showed multidrug resistance belonged to the MDR level, i.e., there were no urine isolates with XDR or PDR. In wound or eschar exudate samples, for every 17 MDR isolates, one was isolated with XDR. In blood culture or catheter samples, for every seven MDR isolates, there was about one isolate with XDR. In bronchial expectoration or secretion samples, for every 20 MDR isolates, one was found with XDR. The two *P. aeruginosa* isolates with PDR were obtained from blood culture or catheter and from bronchial expectoration or secretion samples.

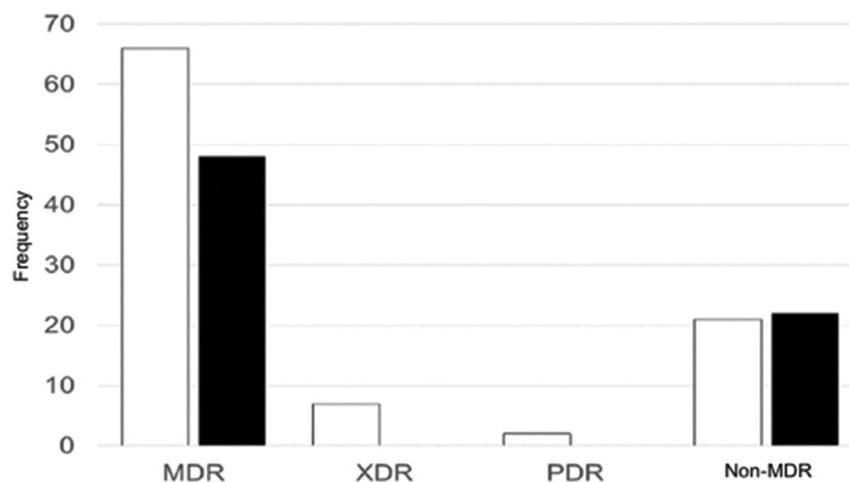


Figure 1. Distribution of isolates by multidrug resistance level and staining. In white color, gram-negative isolates; in black color, gram-positive isolates. MDR: multidrug-resistant; PRD: pan-resistant; XDR: with extended resistance.

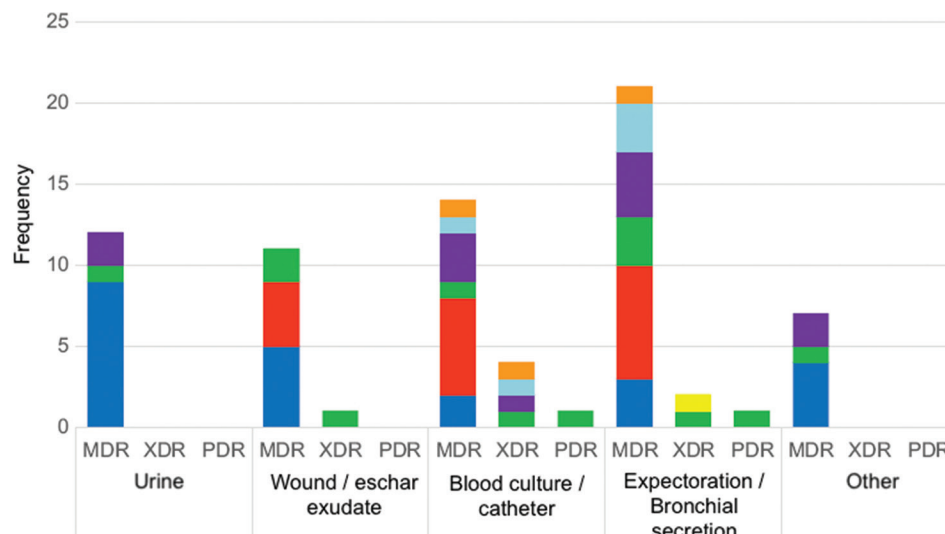


Figure 2. Most common resistant microorganisms and their distribution according to sample origin: *Escherichia coli* (dark blue), *Klebsiella pneumoniae* (purple), *Pseudomonas aeruginosa* (green), *Staphylococcus epidermidis* (red), *Acinetobacter baumannii* (orange), *Klebsiella aerogenes* (light blue) and *Burkholderia cepacia* complex (yellow). MDR: multi-resistant; PDR: pan-resistant; XDR: with extended resistance.

Figure 2 shows the five most common species at each multidrug resistance level. In urine, as already mentioned, all multidrug-resistant isolates showed MDR and all corresponded to *E. coli*. In the samples obtained from wound or eschar exudate, the most commonly isolated multidrug-resistant strains with MDR level were *E. coli* (five isolates) and *S. epidermidis* (four isolates); there was only one isolate with XDR, which corresponded to *P. aeruginosa*, and there were no PDR isolates. The isolates obtained from blood culture or catheter were the ones that showed

more variety; *E. coli* continued to be among the most common bacteria with MDR (two isolates), but *S. epidermidis* was most frequently isolated (6 isolates), and the MDR isolates that followed in frequency included *P. aeruginosa* (one isolate), *K. pneumoniae* (one isolate), *K. aerogenes* (one isolate), and *Acinetobacter baumannii* (one isolate). Finally, as previously mentioned, one of the two *P. aeruginosa* PDR isolates came from a blood culture or catheter sample and the other one, from expectoration or bronchial secretion. In the latter fluid, the bacterial profile resembled, in

Table 3. Distribution of isolates by species and resistance level

Species	Gram stain	Frequency	Resistance level			
			MDR	XDR	PDR	Non-MDR
<i>Escherichia coli</i>	-	29	23	0	0	6
<i>Staphylococcus epidermidis</i>	+	22	17	0	0	5
<i>Pseudomonas aeruginosa</i>	-	18	8	3	2	5
<i>Enterococcus faecalis</i>	+	14	7	0	0	7
<i>Klebsiella pneumoniae</i>	-	14	11	1	0	2
<i>Staphylococcus aureus</i>	+	10	7	0	0	3
<i>Staphylococcus hominis</i>	+	9	5	0	0	4
<i>Staphylococcus haemolyticus</i>	+	7	6	0	0	1
<i>Klebsiella aerogenes</i>	-	5	4	1	0	0
<i>Enterobacter cloacae</i>	-	4	2	0	0	2
<i>Acinetobacter baumannii</i>	-	4	2	1	0	1
<i>Klebsiella oxytoca</i>	-	3	2	0	0	1
<i>Stenotrophomonas maltophilia</i>	-	3	3	0	0	0
<i>Proteus mirabilis</i>	-	2	1	0	0	1
<i>Bacillus cereus</i>	+	2	2	0	0	0
<i>Pseudomonas putida</i>	-	2	1	0	0	1
<i>Streptococcus agalactiae</i>	+	2	1	0	0	1
<i>Serratia liquefaciens</i>	-	1	1	0	0	0
<i>Alcaligenes faecalis</i>	-	1	1	0	0	0
<i>Enterobacter aerogenes</i>	-	1	1	0	0	0
<i>Serratia marcescens</i>	-	1	1	0	0	0
<i>Staphylococcus lentus</i>	+	1	1	0	0	0
<i>Staphylococcus cohnii</i>	+	1	1	0	0	0
<i>Pseudomonas oryzae</i>	-	1	0	0	0	1
<i>Citrobacter freundii</i>	-	1	0	0	0	1
<i>Staphylococcus schleiferi</i>	+	1	0	0	0	1
<i>Providencia rettgeri</i>	-	1	1	0	0	0
<i>Achromobacter spp.</i>	-	1	1	0	0	0
<i>Enterococcus faecium</i>	+	1	1	0	0	0
<i>Salmonella spp.</i>	-	1	1	0	0	0
<i>Burkholderia cepacia complex</i>	-	1	0	1	0	0
<i>Pantoea agglomerans</i>	-	1	1	0	0	0
<i>Sphingobacterium multivorum</i>	-	1	1	0	0	0

MDR: multidrug-resistance; PDR: pan-resistance; XDR: extended resistance.

composition, that of blood culture or catheter; seven *S. epidermidis* isolates, four *K. pneumoniae* isolates and three *E. coli*, *P. aeruginosa* and *K. aerogenes*

isolates, each, were found. Among the most common isolates with XDR obtained from expectoration or bronchial secretion, *P. aeruginosa* and members of

Table 4. Distribution of isolates by department in charge of admission and level of resistance

Department	No. of isolates	MDR isolates n (%)	XDR isolates (%)	PDR isolates n (%)	Total MDR+XDR+PDR n (%)	Total non-MDR n (%)
Surgery	23	17 (74)	0	0	17 (74)	6 (26)
Internal medicine	30	16 (53)	0	0	16 (53)	14 (47)
Gynecology and obstetrics	5	4 (80)	0	0	4 (80)	1 (20)
Pediatrics	5	2 (40)	0	0	2 (40)	3 (60)
Neonatal intensive care unit	31	19 (61)	3 (10)	2 (6)	24 (77)	7 (23)
Intensive care unit	72	56 (78)	4 (5)	0	60 (83)	12 (17)

MDR: multidrug-resistant; PDR: pan-resistant; XDR: with extended resistance.

the *B. cepacia* complex were identified. As supplementary material, Appendix 1 details all isolated microorganisms by species, the sample they were obtained from and the level of resistance (MDR, XDR or PDR).

To explore the magnitude of the multidrug resistance problem at different hospital sections, isolates were classified according to department and multidrug-resistance level (Table 4). Between 53% and 83% of isolates with some level of multidrug-resistance were found at different departments, with the intensive care unit (83.3%), the gynecology and obstetrics department (80%), and the surgery department (73.9%) being the most affected in terms of the proportion of isolates with multidrug-resistance. The neonatal intensive care unit had bacterial isolates with all three levels of multidrug-resistance; the two PDR *P. aeruginosa* strains were isolated from this department (Tables 3-4). There were no bacterial isolates originating from the emergency department.

Discussion

Bacterial resistance is a highly significant problem of global importance that requires surveillance and continuous control for its containment. The WHO has implemented strategies^{12,13} that are essential for actions to be directed. The results of this study show that, as it occurs all over the world¹⁴⁻¹⁶, multidrug-resistance in isolates from our hospital affects both gram-positive and gram-negative microorganisms (Fig. 1). As in other studies¹⁷, *E. coli* and *Klebsiella* sp. were among the most common multidrug-resistant gram-negative microorganisms, and *Pseudomonas* ranked second in terms of frequency among gram-negative multidrug-resistant organisms, and in

multidrug-resistance extension it ranked first, since two clinical isolates had PDR. As for gram-positive microorganisms, *S. epidermidis* was the most frequently isolated multidrug-resistant bacterium, unlike other studies¹⁷ in which *S. aureus* is usually ranked first; 17 of the 22 *S. epidermidis* clinical isolates in this sample were MDR, which is to be considered given its potential to participate in nosocomial infections and biofilm formation. *E. faecalis* was also often isolated, and 50% of these isolates were MDR.

It is alarming to observe that only one out of every four clinical isolates from this sample does not have multidrug-resistance (44 of 166 isolates) (Fig. 1), and that of the 33 identified species only three did not have any level of multidrug-resistance (Table 3). This fact indicates widespread resistance and confirms the seriousness of the problem in the northern region of the country¹⁷.

The multidrug-resistance analysis according to the type of fluid indicates that the problem is more complex and serious in patients with blood infections (or at least in a catheter) or respiratory tract infections, since it was in these fluids that the highest percentage of multidrug-resistant isolates of each level (MDR, XDR and PDR), the greatest variety of bacteria and both PDR isolates were found.

As for hospital departments, the intensive care, gynecology and obstetrics, and surgery departments require attention due to the high proportion of bacterial isolates with multidrug resistance in clinical samples from patients of these departments; in addition, the neonatal intensive care department requires special attention for being the source of PDR isolates.

The obtained information will allow the implementation of measures aimed at the departments with the greatest multidrug resistance problems and focus on

appropriate use of antibiotics in this hospital for the treatment of infectious diseases caused by the microorganisms that were found to have higher multidrug-resistance, both in terms of frequency and severity. A study of other factors possibly involved is underway.

Conclusions

In our study, multiple resistance to antibacterial drugs encompasses up to 75% of clinical isolates and reaches a PDR extension in 1.2% of cases. The most common multidrug-resistant isolates were *E. coli*, *P. aeruginosa*, *S. epidermidis* and *K. pneumoniae*; these species, and the intensive care, surgery, gynecology and obstetrics departments, and the neonatal intensive care unit are priority areas for the study of risk factors and interventions' implementation.

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Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this research.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors declare that there are no conflicts of interest relevant to the performance of this work.

Appendix

Additional material can be accessed in the Appendix, available at: 10.24875/CIRUE.M23000348.

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Appendix

Isolated microorganisms classified according to species, fluid of origin and multidrug-resistance level

Microorganisms	Urine			Wound/eschar exudate			Blood culture/catheter			Expectoration/bronchial secretion			Other**							
	MDR	XDR	PDR	Non-MDR	MDR	XDR	PDR	Non-MDR	MDR	XDR	PDR	Non-MDR	MDR	XDR	PDR	Non-MDR				
<i>Escherichia coli</i>	9	0	0	4	5	0	0	0	2	0	0	0	3	0	0	1	4	0	0	1
<i>Staphylococcus epidermidis</i>	0	0	0	1	4	0	0	0	6	0	0	2	7	0	0	2	0	0	0	0
<i>Pseudomonas aeruginosa</i>	1	0	0	0	2	1	0	2	1	1	1	1	3	1	1	0	1	0	0	2
<i>Enterococcus faecalis</i>	1	0	0	1	2	0	0	1	1	0	0	3	2	0	0	0	1	0	0	2
<i>Klebsiella pneumoniae</i>	2	0	0	0	0	0	0	0	3	1	0	1	4	0	0	1	2	0	0	0
<i>Staphylococcus aureus</i>	0	0	0	0	2	0	0	1	2	0	0	1	3	0	0	1	0	0	0	0
<i>Staphylococcus hominis</i>	0	0	0	1	0	0	0	1	2	0	0	1	3	0	0	1	0	0	0	0
<i>Staphylococcus haemolyticus</i>	0	0	0	0	1	0	0	0	3	0	0	1	1	0	0	0	0	0	0	0
<i>Klebsiella aerogenes</i>	0	0	0	0	0	0	0	0	1	1	0	0	3	0	0	0	0	0	0	0
<i>Enterobacter cloacae</i>	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0
<i>Acinetobacter baumannii</i>	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	1	0	0	0	0
<i>Klebsiella oxytoca</i>	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
<i>Stenotrophomonas maltophilia</i>	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
<i>Proteus mirabilis</i>	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bacillus cereus</i>	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
<i>Pseudomonas putida</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
<i>Streptococcus agalactiae</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
<i>Serratia liquefaciens</i>	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
<i>Alcaligenes faecalis</i>	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
<i>Enterobacter aerogenes</i>	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
<i>Serratia marcescens</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(Continues)

Isolated microorganisms classified according to species, fluid of origin and multidrug-resistance level (continued)

Microorganisms	Urine			Wound/eschar exudate			Blood culture/catheter			Expectoration/bronchial secretion			Other*			
	MDR	XDR	PDR	Non-MDR	MDR	XDR	PDR	Non-MDR	MDR	XDR	PDR	Non-MDR	MDR	XDR	PDR	Non-MDR
<i>Staphylococcus lentus</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
<i>Staphylococcus cohnii</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
<i>Pseudomonas oryzihabitans</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
<i>Citrobacter freundii</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
<i>Staphylococcus schleiferi</i>	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
<i>Providencia rettgeri</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Achromobacter</i> spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>Enterococcus faecium</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Salmonella</i> spp.	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
<i>Burkholderia cepacia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pantoea agglomerans</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
<i>Sphingobacterium multivorum</i>	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0

MDR: multidrug-resistance; PDR: pan-resistance; XDR: extended resistance.

*Cerebrospinal, pleural or peritoneal fluid.