



# Are we correctly targeting the research on disinfection of antibiotic-resistant bacteria (ARB)?

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## ABSTRACT

Antibiotic-resistant bacteria (ARB) is one of the biggest public health challenges of our time since causes thousands of human deaths per year. ARB are bacteria which acquire resistance over time through different mechanism and no longer respond to antibiotics. Numerous bacteria and hydrophilic antibiotics are mainly excreted in patients' urine from sanitary facilities that are directly discharge into municipal sewers. The inefficiency of conventional wastewater treatment plants (WWTPs) for killing ARB leads to their spread in the natural water sources. In this work, a statistical study of the main microorganisms contained in patients' urines with urinary tract infections (UTIs) was analysed using the data supplied from the microbiology and parasitology service in the university hospital complex of Albacete (Spain). A total of 14,368 urine samples were analysed on five hospital units (geriatrics, haematology, oncology, reanimation, and intensive care unit (ICU)) from 2014 to 2018. A chi-square ( $\chi^2$ ) study demonstrated the female prevalence to suffer UTIs in geriatrics, oncology and reanimation units. In addition, the analysis of ARB producing extended spectrum beta-lactamases (ARB<sub>ESBLs</sub>), carbapenemase-producing bacteria (ARB<sub>CPB</sub>) and methicillin-resistant *Staphylococcus aureus* (ARB<sub>MRSA</sub>) were also evaluated. Finally, the literature related to the disinfection of the main ARB previously found in hospital urine was analysed to evaluate the importance given to ARB from an environmental scientific viewpoint.

## 1. Introduction

Since the beginning of 2020, the outbreak of COVID-19 has caused more than 3.12 million deaths worldwide in just over a year. Early advances in vaccines development help to control the pandemic, however, the proliferation of new variants may threaten the effectiveness of vaccines to deal with the virus (Mahase, 2020; To et al., 2020). This important epidemic has also helped to highlight another important health problems which need for urgent actions: the rapid global spread of antibiotic resistance bacteria (ARB) since the treatment of serious diseases with antibiotics is becoming more difficult or nearly impossible. Novel broad-spectrum antibiotics are continuously being developed to kill ARB, but the occurrence of new strains hampers their effectiveness (Fair and Tor, 2014). For this reason, ARB has been classified by the

World Health Organization (WHO), as one of the top 10 global public health threats facing humanity (Organization, 2014; Tacconelli et al., 2018).

Antibiotics belonging to the beta-lactam family are broadly used for the treatment of severe bacterial infections such as urinary tract infections (UTIs), pneumonia, or intra-abdominal infections (Medeiros, 1997). This family comprises all type of penicillins, cephalosporins, cephamycins, monobactams, carbapenems, most of which, kill bacteria by inhibiting bacterial wall synthesis (Queenan and Bush, 2007; Sneader, 2005). An alarming rise in the resistance to beta-lactams in recent years is causing great concern in the scientific community (Patel and Bonomo, 2011). Beta-lactam resistance is common, but not exclusive, to *Enterobacteriaceae*, due to the expression of beta lactamase enzymes (Delgado-Valverde et al., 2013). Specifically, significant

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nosocomial outbreaks are mainly identified among *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) in sanitary facilities (Cantón et al., 2012).

*E. coli* is one of the main intestinal bacterium causing gastroenteritis, UTIs or neonatal meningitis (Tenailon et al., 2010). This gram-negative, coliform bacillus has been extensively studied in the scientific literature for its virulent and antibiotic resistance strains (Cruz-López et al., 2020; Rangel et al., 2005; Troeger et al., 2017). Bala et al. have recently reported the prevalence of beta-lactam producing *E. coli* from hospitalized patients of a sanitary complex in India. 470 samples were analysed and 244 (51.9%) were resistant to cefoxitin, confirming the high prevalence of antibiotic-resistant strains in *E. coli* (Bala et al., 2020). Antimicrobial use, prolonged hospitalization, and interventions were associated risk factors for antibiotic-resistant *E. coli* dissemination. Similarly, *K. pneumoniae* is a gram negative, rod-shaped bacterium, commonly found in mouth, skin and intestines (Ryan and Ray, 2004). Pneumonia, UTIs, wound infections or respiratory tract infections are included in the range of clinical diseases derived from *K. pneumoniae* (Farrell et al., 2003; Podschun and Ullmann, 1998). An antibiotic-resistant strain, *K. pneumoniae* carbapenemases (KPCs) is of particular relevance, since it efficiently hydrolyses most beta-lactam antibiotics, exhibiting high mortality rates (Munoz-Price et al., 2013; Papp-Wallace et al., 2010).

The real danger posed by ARB and their attributable mortality in the European Union (EU) and the European Economic Area (EEA) have been evaluated by Cassini et al. (2019). From European Antimicrobial Resistance Surveillance Network (EARS-Net) data collected during 2015, they estimated 671,689 infections with ARB which accounted for around 33,110 (4.93%) attributable deaths. They also highlighted four ARB with the largest effect on health in the study: *E. coli*, *K. pneumoniae*, *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*).

The high presence of antibiotic-resistant strains combined with the large number of reported cases of ARB derived infections and deaths raises the necessity to gain insight into the clinical epidemiology of the ARB expansion of each geographical area. In order to develop treatment technologies capable to destroy these ARB, it is extremely important to know more about them and, particularly, on the extension of the problem associated with each particular ARB. For this, it is important to know how the effluents of hospitals are, because this is the point in which the highest concentrations of these ARBs can be found. In this paper, an analysis of the main ARB contained in hospital urines has been carried out based on the data supplied by the University Hospital Complex of Albacete, Spain (CHUA) as model of sanitary facility. The CHUA depends on the health service of Castilla-La Mancha (SESCAM) and is integrated by the General University Hospital of Albacete, the University Hospital of Perpetuo Socorro and the Mental Health Care Centre of Albacete. It is equipped with 752 beds, representing a total of 2.54 beds per 1000 inhabitants. Data on positive urine pathogens have been provided by the Microbiology and Parasitology Service of CHUA. The patients under study have a clinical diagnosis of urinary tract infection (UTI) and belong to different hospital units. This will allow to evaluate the occurrence and fate of ARB in a real environment as well as the problematic associated with ARB in a hospital complex. Additionally, this will provide valuable information for the development of new renewable and sustainable disinfection technologies.

## 2. Materials and methods

### 2.1. Classification of antibiotic-resistant bacteria in urine samples

The ARB percentages of *K. pneumoniae*, *E. coli* and *S. aureus* were calculated from the data provided by the University Hospital Complex of Albacete, Spain (CHUA). 4,453 urines were analysed finding that 426 samples were positive for *K. pneumoniae*, of which 143 (33.56%) presented antibiotic resistance. From these 143 ARB samples, 138 (96.51%) produced extended spectrum beta-lactamases (ESBLs) and, 5 (3.49%)

were carbapenemase-producing bacteria (CPB). Furthermore, 1,866 samples were positive for *E. coli*, but only 269 were ARB (14.41%). From these samples, 268 (99.63%) were ARB<sub>ESBL</sub> and, 1 (0.37%) was ARB<sub>CPB</sub>. Finally, *S. aureus* were positive in 65 urine samples, where 32 (49.23%) presented methicillin resistant (MRSA). Table 1 summarizes the urine samples analysed from CHUA.

### 2.2. Analysis of scientific literature related to antibiotic-resistant bacteria

A literature search was conducted using SCOPUS database in order to determine the importance given by scientific community to the disinfection of the main ARB found in hospital urines. For this purpose, the following sets of keywords were searched: “disinfection water *Escherichia coli*”, “disinfection wastewater *Escherichia coli*”, “disinfection hospital wastewater *Escherichia coli*”, “disinfection water *Klebsiella pneumoniae*”, “disinfection wastewater *Klebsiella pneumoniae*”, “disinfection hospital wastewater *Klebsiella pneumoniae*”, “disinfection water *Staphylococcus aureus*”, “disinfection wastewater *Staphylococcus aureus*” and “disinfection hospital wastewater *Staphylococcus aureus*”.

## 3. Occurrence of ARBs in urine from a hospital complex

Urinary tract infections (UTIs) are some of the most common infections in hospitals and represent a significant cause of morbidity of males and females of all ages (Stamm and Norrby, 2001). Severe health problems associated with UTIs include pyelonephritis with sepsis, renal damage, pre-term birth and complications caused by frequent antimicrobial use (Flores-Mireles et al., 2015). Several risk factors are associated with UTIs, including female gender, sexual activity, vaginal infection, diabetes, obesity or genetic susceptibility among others (Foxman, 2002, 2013). For this reason, the prevalence of gender in the presence of pathogens in urine has been considered in this study.

There are many areas in a sanitary complex where patients can present UTIs and, consequently, urines analysed come from different units of CHUA: geriatrics, haematology, oncology, reanimation, and intensive care unit (ICU). A total of 14,368 urine samples were analysed and 4,453 (30.99%) were positive for the presence of pathogens from 2014 to 2018. Within these positive urines (PUs), 2,874, 286, 371, 421 and 528 correspond to geriatrics, haematology, oncology, reanimation and ICU, respectively. The distribution of these PUs over the 5-year period studied and the differences in infected patients between males

**Table 1**  
Urine samples analysed.

TOTAL URINE SAMPLES (4,453)	<i>K. pneumoniae</i> (426 samples) (9.57%)	ARB (143 samples) (33.56%)	ESBL (138 samples) (96.51%) CPB (5 samples) (3.49%)
		No ARB (283 samples) (66.44%)	
	<i>E. coli</i> (1,866 samples) (41.90%)	ARB (269 samples) (14.41%)	ESBL (268 samples) (99.63%) CPB (1 samples) (0.37%)
		No ARB (1,597 samples) (85.58%)	
	<i>S. aureus</i> (65 samples) (1.46%)	ARB (32 samples) (49.23%)	MRSA (32 samples) (100%)
		No ARB (33 samples) (50.77%)	
	Others (2,096 samples) (47.07%)		

and females are shown in Table 2.

As previously commented, UTIs have a gender factor, making women be more likely to develop them. This is supported by Table 2, where during the 5-year period, 62.36% of PUs correspond to females. The number of PUs increases over the years, peaking in 2018, which could be attributed to the ageing of the population. As expected, geriatrics is more affected by this phenomenon with 473 PUs registered in 2014 and were almost doubled (836) 5 years later. PUs from geriatrics represent 64.54% of all PUs which could be related to the particularities that characterize patients from this unit such as being elderly people (over 65) or presenting several pathologies with greater incidence of illness in the same individual (Chenoweth et al., 2014; Samaras et al., 2010; Schmiemann et al., 2010). PUs from haematology, oncology, reanimation and ICU are within the range 50–121 samples for all the years analysed, being higher in the last years. Differences in the age of patients of each unit are also noticeable. Specifically, elderly patients  $\geq 65$  years represent 99.23, 63.64, 54.18, 57.72 and 53.79% of the total PUs of geriatrics, haematology, oncology, reanimation and ICU, respectively. These data reveal that the potential for developing an UTI could be directly related to the age and gender of the patient.

Many studies focused on the epidemiology of UTIs have demonstrated that UTIs are caused by a wide range of pathogens, including gram-negative and gram-positive bacteria, and yeasts (Chen et al., 2013; Jacobsen et al., 2008; Ronald, 2002). Despite the above-mentioned microorganisms are found in greater proportion in PUs, the number of pathogens that can be found in a smaller percentage is quite extensive. In this case of study, only pathogens that represent more than 1.00% of the total pathogens have been included. Likewise, it is important to mention that two or more pathogens can result positive for a single urine sample analysed, being 5,036 the number of pathogens for the 4,453 PUs reported in the study. The distribution of pathogens in the different hospital units during the 5-year period is represented in Fig. 1.

*Escherichia coli* (*E. coli*) was the most significant pathogen in the PUs of all hospital units, being present in 1866 patients, 1365 women and 501 men. Specifically, it ranges from 20.00 to 50.00%, with a mean value of 37.05% of the total pathogens in the different hospital units over the years. This value is slightly lower than that previously reported in literature (65.00–75.00%) (Flores-Mireles et al., 2015). Nonetheless, other studies have demonstrated that the microbiology of UTIs depends on its pathology. For instance, in a review of multicenter data on Catheter-associated Urinary Tract Infections (CAUTI), *E. coli* was only present in 23.90% of PUs between 2011 and 2014 (Weiner et al., 2016).

Regarding other gram-negative bacteria, *Klebsiella pneumoniae* (*K. pneumoniae*) (3.80–15.00%), *Pseudomonas aeruginosa* (*P. aeruginosa*) (0.00–12.10%) and *Proteus mirabilis* (*P. mirabilis*) (0.00–6.20%) are the most representative ones, considering all hospital units. Gram-positive bacteria are mainly represented by *Enterococcus faecalis* (*E. faecalis*), the most significant pathogen of the family of *Enterococcus* spp., ranging from 3.80 to 16.00% in all PUs. The genus of *Staphylococcus* is poorly represented by *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*), both with low prevalence percentages. The presence of *Staphylococcus saprophyticus* (*S. saprophyticus*), which was previously introduced as the main specie of *Staphylococcus* spp. in UTIs, was below 1.00% in PUs, so it was neglected. Group *B Streptococcus* is weakly constituted by *Streptococcus agalactiae* (*S. agalactiae*) with only

presence in haematology and oncology. Finally, *Candida* spp. represents the most predominant yeasts family in all hospital services. *Candida albicans* (*C. albicans*) is the species found in the highest percentage, followed by *Candida glabrata* (*C. glabrata*) and *Candida parapsilosis* (*C. parapsilosis*).

Paying special attention to each hospital unit individually, geriatrics seems to be the unit with the least variability in the ranges of pathogens over the years. In this hospital unit, *E. coli* was the most common pathogen ranging from 34.10 to 44.10% followed by *E. faecalis* (10.10–13.10%), *K. pneumoniae* (6.20–11.00%), *C. albicans* (6.10–7.80%) and *P. mirabilis* (4.10–6.20%). Similar results have been recently reported in Denmark, in a study with elderly patients  $\geq 60$  years where 15,242 cases of nosocomial UTIs were registered. The most common pathogens found were *E. coli*, *E. faecalis*, *K. pneumoniae* and *Enterococcus faecium* (*E. faecium*), present in 58.00, 28.00, 9.00 and 5.00% of the total cases (Leihof et al., 2019). On the other hand, it is worth noting the elevated presence of *E. coli* in the unit of haematology, where in most of the years the percentage of prevalence were higher than 40.00%. Conversely, in the unit of oncology, the presence of this pathogen has decreased over the years, from 46.60% in 2014 to 24.50% in 2018. Reanimation and ICU units have an important prevalence of *C. albicans* (13.50–26.80% and 7.50–17.40%, respectively), being the most significant pathogen in PUs after *E. coli*. These results are supported by the National Nosocomial Infection Surveillance system reports where *C. albicans* is reported as the second cause of nosocomial UTIs in ICUs (Fridkin et al., 1997).

To evaluate the prevalence of the total gram-negative and gram-positive bacteria, and yeasts in PUs from different hospital units, Fig. 2a shows the percentage distribution of each type of microorganism over the 5-year period in the different hospital units.

Results clearly depict the dominance of gram-negative pathogens in PUs with a presence between 40.30 and 70.70% of the total pathogens for all the units studied. This agrees the *E. coli* prevalence in UTIs reported in the literature (Flores-Mireles et al., 2015) and, it is also supported by several studies related to the pathology of gram-negative organisms in nosocomial UTIs (Gaynes and Edwards, 2005; Peleg and Hooper, 2010). Regarding the distribution of gram-positive bacteria, their presence is within the range of 9.87–35.84% in all PUs from the different hospital units, being clearly lower than the percentages registered for gram-negative bacteria. However, it is worth noting the highest percentages of gram-positive bacteria exhibited in the PUs from the oncology unit (35.84–17.64%), which can be related with the higher risk of oncology patients of being colonized or infected by microorganisms of *Enterococcus* genus (Mutter et al., 2013). On the other hand, the prevalence of yeasts is higher in reanimation and ICU units which agrees with other studies reported in literature (Álvarez-Lerma et al., 2003; Sardi et al., 2013; Vincent et al., 2009). For instance, a study at a University Hospital in Turkey showed that the presence of *Candida* spp. was predominant for the Anaesthesiology and Reanimation Unit (Ece, 2014). Besides, patients hospitalized in ICUs are at great risk for developing nosocomial fungal infections presenting common risk factors. Some of them act primarily by inducing immunosuppression (e.g., corticosteroids, chemotherapy, malnutrition, malignancy, and neutropenia) while others primarily provide a route of infection (e.g., extensive burns, indwelling catheter), and some act in combination

Table 2

Distribution of PUs in each hospital unit from 2014 to 2018 in CHUA. T: Total, F: Female (%), M: Male (%).

	PUs 2014			PUs 2015			PUs 2016			PUs 2017			PUs 2018		
	T	F (%)	M (%)	T	F (%)	M (%)	T	F (%)	M (%)	T	F (%)	M (%)	T	F (%)	M (%)
GERIATRICS	473	71.04	28.96	482	70.54	29.46	457	64.77	35.23	599	69.62	30.38	836	65.07	34.93
HAEMATOLOGY	50	78.00	22.00	70	40.00	60.00	52	61.54	38.46	57	63.16	36.84	57	64.91	35.09
ONCOLOGY	51	50.98	49.02	70	57.14	42.86	83	53.01	46.99	85	57.65	42.35	82	37.80	62.20
REANIMATION	74	43.24	56.76	76	46.05	53.95	72	56.94	43.06	78	43.59	56.41	121	46.28	53.72
ICU	91	51.65	48.35	120	57.50	42.50	91	54.95	45.05	108	44.44	55.56	118	59.32	40.68

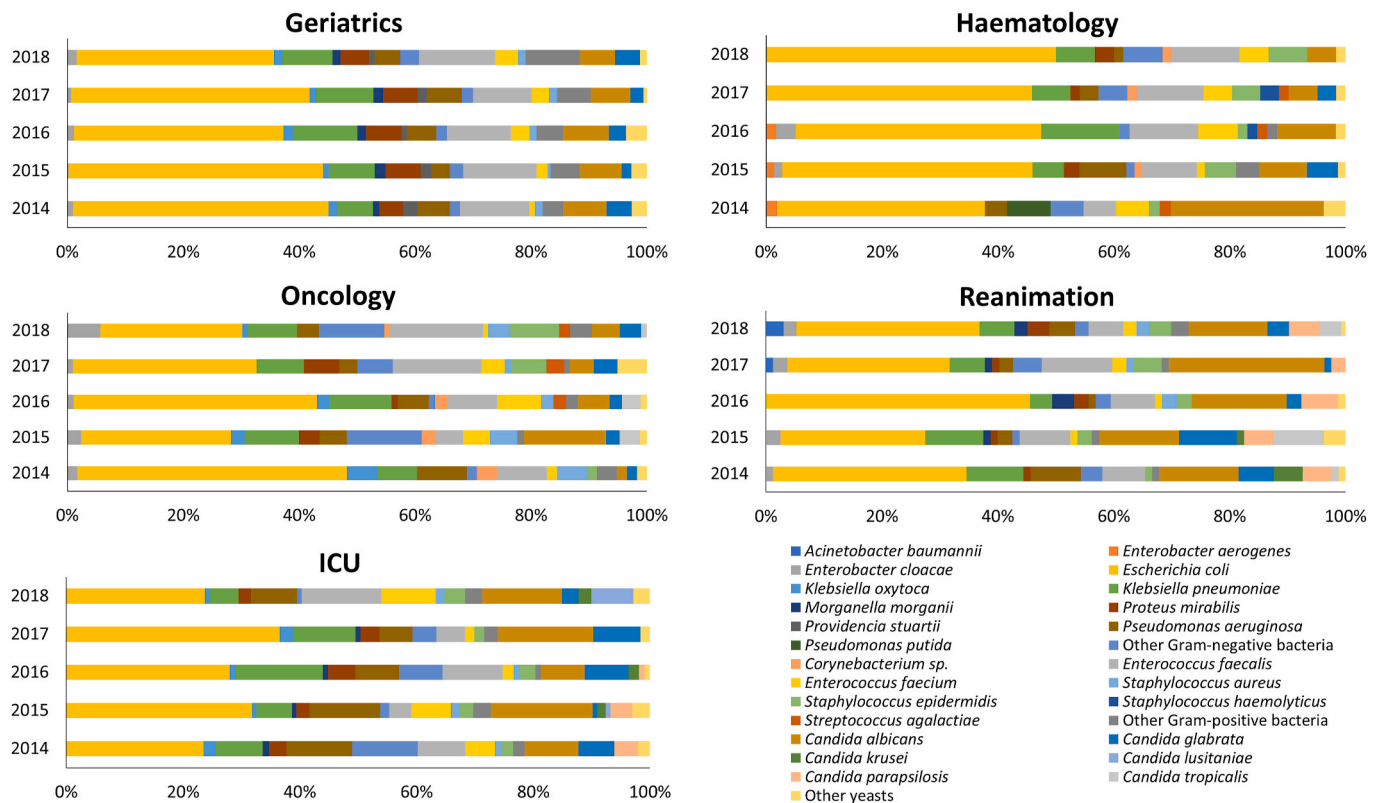


Fig. 1. Percentage of positive microorganisms, representing more than 1.00% of total PUs, in the different hospital units during the period 2014–2018.

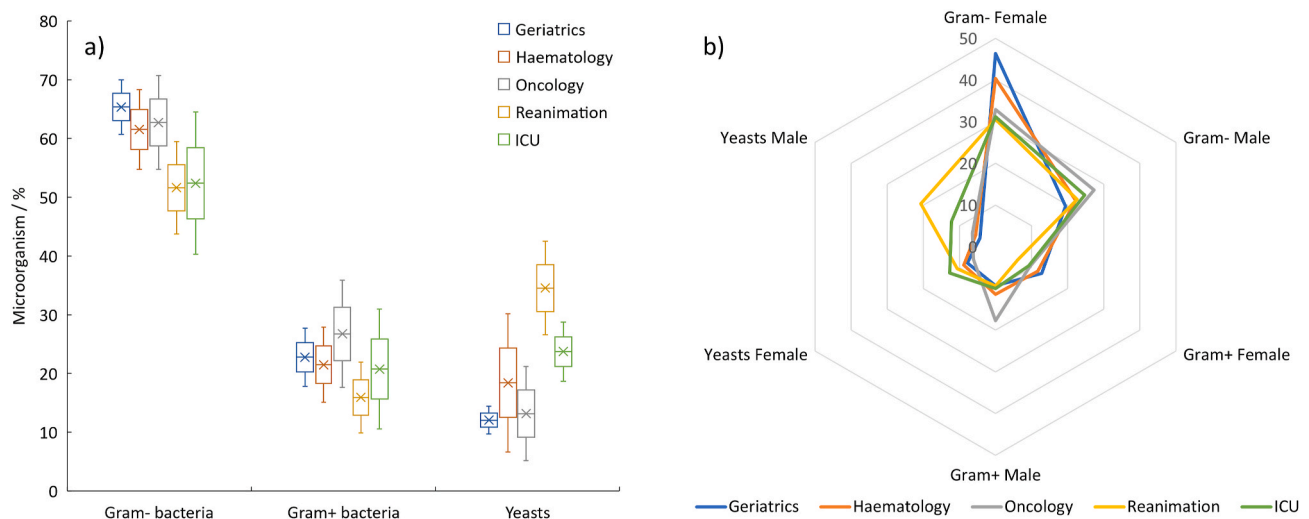


Fig. 2. Distribution of total microorganisms corresponding to gram-, gram + bacteria and Yeasts for each hospital unit during the years 2014–2018. (a) Maximum, minimum, and mean value (\*); (b) Gender prevalence.

(Fridkin and Jarvis, 1996). This could also explain the lower percentages of gram-negative bacteria registered in these units in comparison with geriatrics, haematology and oncology.

As stated above, women are more likely to suffer UTIs and, for this reason, the influence of gender in the presence of gram-negative and gram-positive bacteria, and yeasts in PUs is represented in Fig. 2b. Furthermore, a chi-square ( $\chi^2$ ) study of the gender prevalence in the different hospital units has been carried out from the data of Fig. 2b. The  $\chi^2$  study is one of the tests belonging to the descriptive statistics applied to the study of two variables. It is a hypothesis test that compares the observed distribution of the data with an expected distribution of the

data. The test uses an approximation of the  $\chi^2$  distribution to evaluate the probability of a discrepancy equal to or greater than that between the data and the expected frequencies according to the null hypothesis. The independence of two variables means that they are unrelated, and therefore one does not depend on the other, nor vice versa (Corder and Foreman; Greenwood and Nikulin, 1996). Results obtained from the  $\chi^2$  study conclude that there is a statistically relevant relationship between the gender and PUs in geriatrics, oncology and reanimation units. On the contrary, there is no such relationship, independent variables, in haematology and ICU. The  $\chi^2$  study establishes for geriatrics, oncology and reanimation units that; 1) if a patient suffers a UTI associated with a



gram-negative bacteria, the probability of being female would be 70.40, 54.70 and 57.40%, respectively, 2) if a patient suffers a UTI associated with a gram-positive bacteria, the probability of being female would be 57.80, 35.00 and 39.40%, respectively, and 3) if a patient suffers a UTI associated with a yeast, the probability of being female would be 64.70, 49.10 and 33.80% for the same units, respectively.

UTIs can be caused by a wide variety of pathogens, however, those with antibiotic resistance involve a big challenge for their elimination. Infections caused by ARB cannot be treated with commonly used clinical antimicrobials, leading to serious health problems and high associated costs (Levy and Bonnie, 2004). Extended spectrum beta-lactamases (ESBLs) are enzymes able to hydrolyse third and fourth generation cephalosporins and monobactams (Paterson and Bonomo, 2005). The epidemiology of ESBLs changes rapidly worldwide and, hence, there is no general overview of these enzymes in UTIs. Specifically, the antibiotic resistance of *E. coli* and *K. pneumoniae* associated to ESBLs is particularly high in some countries of the European Union, where UTIs can no more be treated with the common antibacterial classes, according to the European Antimicrobial Resistance Surveillance Network (EAR-S-Net). Therefore, ESBLs represent a public health concern due to the high prevalence in these common pathogens (Europe, 2014; Mazzariol et al., 2017). In the case of study, the percentage of *K. pneumoniae* and *E. coli* producing ESBLs (ARB<sub>ESBL</sub>) in PUs from the different hospital units of CHUA over the 5-year period is represented in Fig. 3a.

Overall, 32.39% of total *K. pneumoniae* in PUs from all hospital units are ARB<sub>ESBL</sub>. The percentage of this bacterium increased gradually throughout the years, from 9.80% in 2014 to 45.30% in 2018, being noticeable the higher percentages of ARB<sub>ESBL</sub> in the reanimation unit in 2016 (66.70%), and in the haematology unit in 2015 (75.00%) and 2018 (100.00%). On the other hand, the presence of *E. coli* producing ESBLs

was much lower than that shown by *K. pneumoniae*, representing just the 14.36% of total *E. coli* in PUs from all hospital units. The presence of this bacterium also showed an increasing tendency through the 5-year period of the study, from 9.60% in 2014 to 16.70% in 2018. These results highlight the greater threat to human health posed by *K. pneumoniae* (ARB<sub>ESBL</sub>) and support the world's growing concern for this bacterium.

Other mechanisms of antibiotic resistance are possible for *E. coli* and *K. pneumoniae*. Specifically, in the late 90s, the carbapenem-resistant *K. pneumoniae* began to emerge and, currently, is the most common carbapenemase detected globally (Bush and Bradford, 2020; Woodford et al., 2004). Carbapenemase-Producing Bacteria (CPB) have a large and shallow active site, allowing these enzymes to accommodate a wide range of  $\beta$ -lactam molecules, including cephalosporins, monobactams, and carbapenems (Ke et al., 2007). Acquired serine carbapenemase enzymes are resistant to most  $\beta$ -lactams and they encode a multitude of other resistance genes, offering multidrug resistance (Pitout et al., 2015). In the present study, the percentage of *K. pneumoniae* and *E. coli* producing CPB (ARB<sub>CPB</sub>) in PUs from the different hospital units over the 5-year period is represented in Fig. 3b.

In this case, 1.17% of total *K. pneumoniae* in PUs from all hospital units were ARB<sub>CPB</sub>. Nonetheless, these data were only obtained from oncology, ICU and reanimation because the presence of ARB<sub>CPB</sub> in geriatrics and haematology was null. Despite the low percentage of ARB<sub>CPB</sub> in all years analysed, this increased up to 25.00% in reanimation unit in 2018. Once again, *K. pneumoniae* presented a higher percentage of resistance than *E. coli*, in which only 1 case out of 1,866 positives (0.05%) were ARB<sub>CPB</sub>. These results reveal that ARB<sub>ESBL</sub> are more widespread than ARB<sub>CPB</sub> and the higher resistance percentages of *K. pneumoniae* point out again the need to control this bacterium.

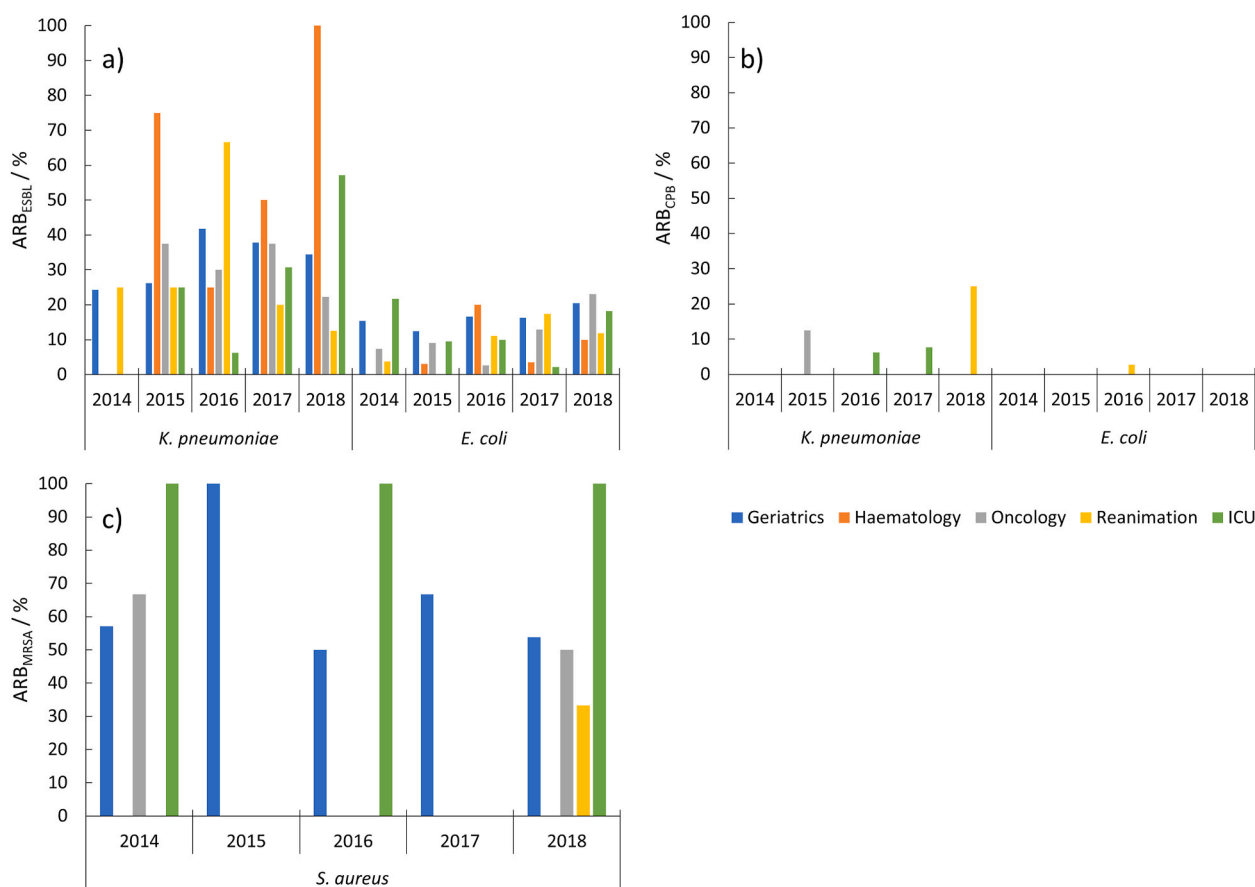


Fig. 3. Evolution of the percentage of ARB in the different hospital units during the period 2014–2018 through different mechanisms. (a) ARB<sub>ESBL</sub>, (b) ARB<sub>CPB</sub>, (c) ARB<sub>MRSA</sub>.

On the other hand, methicillin-resistant *S. aureus* (MRSA) strains are common causes of nosocomial infections in hospital facilities, including ITUs (Hidron et al., 2008; Weiner et al., 2016). The prevalence of MRSA strains has significantly increased over the years. In fact, the spread of MRSA infections from healthcare facilities to various community settings has raised considerable concern in recent decades (Lim et al., 2019). The *mecA* gene is responsible for carrying resistance to methicillin. This gene has a large heterologous mobile genetic element called Staphylococcal Cassette Chromosome *mec* (SCCmec) that promotes *S. aureus* resistance to Methicillin. The increasing emergence of resistance to currently available antimicrobial agents among MRSA strains has limited the choice of therapeutic options and is becoming a serious threat to public health (Sabzeali et al., 2019). Fig. 3c shows the percentage of *S. aureus* producing MRSA (ARB<sub>MRSA</sub>) in PUs from the different hospital units over the 5-year period. A total of 65 samples were positive for *S. aureus*, of which, 32 presented methicillin resistance. Although MRSA strains are less common, their occurrence is much higher than those of ESBL (Fig. 3a) and CPB (Fig. 3b) in the CHUA since almost 50.00% of analysed cases exhibited antibiotic resistance. Specifically, the presence of ARB<sub>MRSA</sub> is most remarkable in geriatrics, reaching percentages of 100.00% in 2015, and in ICU, where values of 100.00% in the years 2014, 2016 and 2018 were achieved. However, no ARB<sub>MRSA</sub> were registered in haematology unit within the years analysed.

In summary, 426 samples were positive for *K. pneumoniae* where a percentage of 33.56% were ARB, being 96.51% ARB<sub>ESBL</sub> and 3.49% ARB<sub>CPB</sub>. Conversely, 1,866 samples were positive for *E. coli*, finding 14.41% as ARB of which 99.63% were ARB<sub>ESBL</sub> and 0.37% ARB<sub>CPB</sub>. Eventually, ARB<sub>MRSA</sub> represent 49.23% of the total *S. aureus* in PUs (65 samples). Thus, ARB represent 9.97% of the total PUs (4,453) reported in the study, 8.81% of the total microorganisms (5,036) reported, and 18.83% of the three bacteria presenting ARB, *K. pneumoniae*, *E. coli*, and *S. aureus* (2,357).

#### 4. Scientific interests in destruction of ARBs

Based on previously data (Fig. 3), *K. pneumoniae*, *E. coli* and *S. Aureus* can be considered as bacteria that pose a higher risk for human health. Furthermore, these ARB and their ARGs have been commonly detected in wastewater, surface water, drinking water biofilms and urban treated wastewater (Rizzo et al., 2013; Schwartz et al., 2003). For this reason, it is necessary to develop novel and efficient technologies that allow to kill these pathogens for avoiding their spread not only in sanitary facilities but also in the environment. In this context, a literature search focused on these specific bacteria and the disinfection technologies proposed for their elimination in three different scenarios (water disinfection, wastewater disinfection and hospital wastewater disinfection) was carried out. This allows us to compare the importance given to the main ARB found in the case study from a sanitary and environmental-technological viewpoint. The search has been carried out using SCOPUS as database and the following keywords (April 30th, 2021): “disinfection”, the type of effluent (“water”, “wastewater” or “hospital wastewater”) and the specific bacteria (“*Escherichia coli*”, “*Klebsiella pneumoniae*” or “*Staphylococcus aureus*”), finding 3,977 documents for water, 987 documents for wastewater and only 33 documents for hospital wastewater. Fig. 4 summarizes the percentage of scientific papers related to the disinfection of each ARB in the different scenarios.

As can be observed, the most studies reported in literature are focused on the removal of *E. coli* followed by *S. aureus* and, finally, *K. pneumoniae*. This is more remarkable in the disinfection of wastewater where scientific papers related to *E. coli* achieve 90% (888 documents). This is an expected outcome because this pathogen is the most common indicator of faecal pollution, and their removal is critical for very important applications such as wastewater reclamation. On the other hand, the research reported on the disinfection of hospital wastewater shows the lowest percentage of documents related to *E. coli* (61%; 20

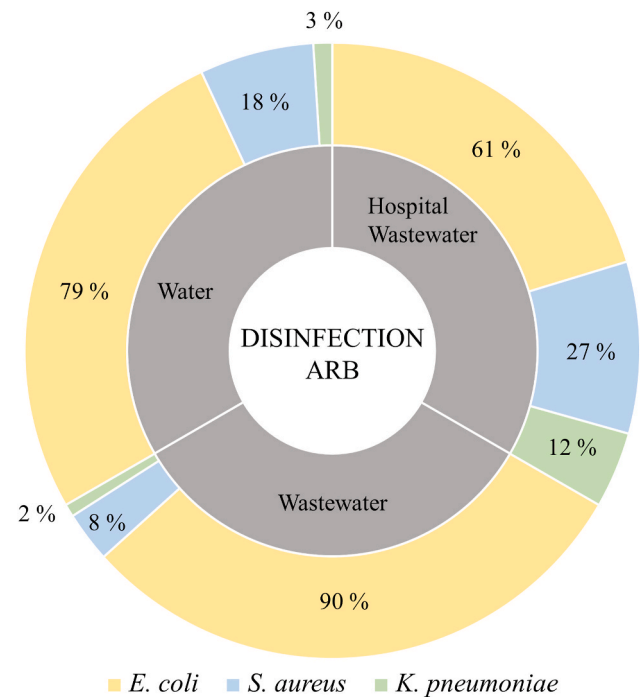


Fig. 4. Literature reported on disinfection of ARB.

documents) in comparison with the disinfection of water (79%; 3,142 documents) and wastewater (90%; 888 documents). In this scenario, the manuscripts related to the removal of *S. aureus* and *K. pneumoniae* are higher, reaching values of 27 (9 documents) and 12% (4 documents), respectively. The studies reported in water and wastewater based on the removal of these bacteria are lower than 20% for *S. aureus* (water: 716 documents; wastewater: 79 documents) and, only 2–3% of all documents for *K. pneumoniae* (water: 119 documents; wastewater: 20 documents). This points out the importance given for these ARB in hospital wastewater by scientists because of the sanitary risks associated to both bacteria. Nonetheless, the research carried out in the disinfection of hospital wastewater is mainly focused on the removal of *E. coli* since is the pathogen found in the highest incidence in urines from the different hospital areas (Fig. 1).

These results reveal that the importance given to the disinfection of the main ARB found in hospital urine by scientific community is not directly related to their hazardousness from a sanitary viewpoint since *K. pneumoniae* can be considered as the main ARB in hospital urine but the research about its disinfection is scarce.

#### 5. Conclusions

*E. coli* was the most significant bacteria found in PUs regardless the hospital unit studied. The UTIs were significantly affected by the age and gender of patients. Gram-negative bacteria (*E. coli*, *K. pneumoniae*, *P. aeruginosa* and *P. mirabilis*) predominate over gram-positive bacteria (*E. faecalis*) and yeasts (*C. albicans*). However, *K. pneumoniae* showed the highest percentages of ARB<sub>ESBL</sub> and ARB<sub>CPB</sub>, confirming its greater hazardousness to human health from a sanitary viewpoint. On the contrary, the research carried out on the disinfection of ARB is mainly related to the removal of *E. coli* since is the pathogen found in the highest proportion in water bodies. The importance given to *K. pneumoniae* as ARB from a sanitary viewpoint does not correspond to the research carried out on the disinfection of ARB by the scientific community because the literature reported is scarce and the likelihood of finding antibiotic resistant is higher in *K. pneumoniae* than in *E. coli*, despite the last one is found in greater extension. Hence, it is necessary to search and to develop novel technologies that allow to remove *K. pneumoniae* ARB

for decreasing the sanitary and environmental impact of the effluents infected with this bacterium.

## CRediT authorship contribution statement

**Miguel Herraiz-Carboné:** Investigation, Formal analysis, Writing. **Salvador Cotillas:** Supervision, Writing, Methodology, Conceptualization. **Engracia Lacasa:** Supervision, Writing, Methodology, Conceptualization. **Caridad Sainz de Baranda:** Formal analysis, Methodology. **Eva Riquelme:** Formal analysis, Methodology. **Pablo Cañizares:** Funding acquisition, Project administration. **Manuel A. Rodrigo:** Supervision, Conceptualization. **Cristina Sáez:** Funding acquisition, Supervision, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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