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ORIGINAL ARTICLE

No global increase in resistance to antibiotics: a snapshot of resistance from 2001 to 2016 in Marseille, France

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Abstract

Since effective empirical antibiotic therapy is a key factor for survival, local antibiotic resistance epidemiology is critical. We aimed to identify current trends in antibiotic resistance for key antibiotics obtained over 16 years (2001–2016) for invasive infections corresponding to empirical treatment in a large hospital centre in Marseille, France.

From January 2014 to December 2016, we have collected all data on antibiotic susceptibility from public laboratory hospitals, and a retrospective analysis was performed on key antibiotics in blood cultures since 2001. A total of 99,932 antibiotic susceptibility testings (ASTs) were analysed, and proportion of pan-drug resistant (PDR = resistant to all antibiotics tested) and extensively drug-resistant (XDR = resistant to all except for two classes) strains were < 0.03 and 0.5%, respectively. Between 2001 and 2016, we found an increase of resistance to third-generation cephalosporins for *E. coli* invasive strains (0% vs 17.8%; $p < 10^{-5}$) and *K. pneumoniae* (8% vs 35.4%; $p = 0.001$) along with a decrease of methicillin-resistant *S. aureus* strains (31% vs 19.8%; $p = 0.006$). Moreover, during the 3-year period, a significant increase of wild-type strains, susceptible to all antibiotics tested, was observed in invasive infections. Regarding bacteraemia involving *Enterobacteriaceae* and *S. aureus*, empirical therapy is effective in > 99% cases. Active epidemiological surveillance is necessary because antibiotic resistance remains unpredictable.

Keywords Antibiotic resistance · Snapshot · Epidemiology · Marseille

Introduction

Resistance to antibiotics is a natural phenomenon that existed even prior to the use of antibiotics in humans. For example, the metallo-β-lactamase enzyme evolved 2 billion years ago before the differentiation into Gram-negative or in Gram-positive bacteria and has been found in humans, different bacteria, and archaea [1]. The resistance to antibiotics evolves over time and can be influenced by the intensive use of antibiotics in humans or in animals, such as for resistance to colistin [2]. However, if the level of resistance is variable between bacterial species, resistance is not cumulative and other factors must be considered [3]. Some reports indicated that antimicrobial resistance will be responsible for the death of more than 10 million people by 2050 worldwide [4]. However, how can we attribute the increase in mortality to multi-drug resistant bacteria (MDR)? It was recently shown that these reports are solely based on mathematical models from previous existing studies [5, 6]. The latter rely on the prevalence of resistance in some countries, which cannot be used as a predictor for the entire trend of antibiotic resistance in a given area, and thus, of the deaths attributable to MDR. As a matter of fact, several results from a recent study emphasising the burden of antibiotic resistant bacteria in Europe are strongly impacted by the situation in Greece and Italy in particular concerning carbapenemase production [7]. Indeed, none of these mortality predictions attributed to MDR bacteria consider "real-life" settings as assessed by surveillance systems. Such extrapolations are unrealistic because one of the parameters, the resistance to antibiotics, is itself unpredictable [8]. The current problem of resistance to antibiotics in Europe concerns Gram-negative bacteria, for which extended spectrum B-lactamase (ESBLs) [9], carbapenemases [10, 11], and more recently, colistin mediate resistance via the *mcr-1* gene coded on plasmids [12], is currently emerging. By contrast, Gram-positive bacteria are not affected. There is no

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general trend for an increase in antibiotic resistance in all bacterial species, but we observed resistance increases or decreases without understanding the reason for this. Current studies on resistance are focused on bacterial groups that become resistant (i.e. Gram-negatives) suggesting that the resistance increases in all pathogenic bacteria. However, in reality, there is a balance to resistance to antibiotics [13]. Moreover, it was clearly demonstrated that mortality due to MDR bacteria is mainly due to inadequate empirical treatment (6 underlining the need of updated guidelines according to local epidemiology). In addition, there are many confounding factors and comorbidities that are associated with mortality and not with antibiotic resistance. Moreover, the definition of MDR bacteria is often confused [14, 15], depending on the geographic region and the different disciplines. In fact, no clear definition exists because it depends on the number of antibiotics tested, which focused on empirical treatment or infections caused by MDR without response to antibiotics [16].

Therefore, local surveillance of the prevalence of resistance to antibiotics commonly used to treat infections is critical to provide the best empirical therapeutic options. In Marseille, two types of automatic epidemiological surveillance systems have been developed. One specific tool is dedicated to the real-time analysis of microbiological data from the various Marseille hospitals [17], and the other is for epidemiological surveillance implemented across the entire southeast area of France [18]. These systems compare the results obtained from clinical microbiology laboratories to a historical database to confirm the presence of a specific abnormal event. For Marseille, the epidemiological surveillance systems can detect an increase in positive samples involving a specific microorganism and abnormal susceptibility patterns [17].

The aim of our study was to identify current trends in antibiotic resistance, specifically examining key antibiotics used for empirical treatment by analysing the local epidemiology of the most common bacteria responsible for infectious diseases in a large hospital centre in Marseille over a 3-year period (January 2014 to December 2016) and to compare this to existing data on the prevalence of resistance in blood cultures from 2001 to 2013.

Materials and methods

Study design

A retrospective analysis was performed from January 2014 to December 2016. In this study, we focused on antibiotic susceptibility testing (AST) of the 15 most common bacteria isolated from public laboratory hospitals in Marseille, France. A total of four university hospitals were included in this study, La Timone Hospital (1500 beds), Conception Hospital (700 beds), Sainte Marguerite Hospital (900 beds), and North Hospital (600 beds). All specimens are grouped and treated in a single laboratory for clinical microbiology.

A second retrospective analysis was performed in our laboratory based on the five most important pathogens, with only results on key antibiotics isolated in blood samples, including *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Staphylococcus aureus* from 2001 to 2014.

Bacteria identification and antibiotic susceptibility testing

Samples received were processed according to standard microbiological procedures.

Isolates included in this study were identified using matrix assisted laser desorption ionisation-time of flight (MALDI-TOF) [19] exclusively. AST was performed using the disc diffusion method and interpreted as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [20] and minimum inhibitory concentrations (MICs) were obtained using the E-test method (Biomérieux, Marcy l'Etoile, France). For several antibiotics/bacteria combinations, which are missing from EUCAST guidelines, French local recommendations were used (i.e. Comité de l'antibiogramme–Société Française de Microbiologie) (Table S1). The number of antibiotics tested ranged from 6 to 12 depending on the bacterial species corresponding to the standard panel. All details of the panel per species are presented in Table 1. The standard panel includes the most common antibiotics tested. For strains resistant to more than three classes of antibiotics, MICs were determined for additional agents depending on the species, including fosfomycin, colistin, minocycline, and tigecycline. Other MICs were determined to definitely categorise susceptibility to carbapenems (i.e. imipenem and ertapenem) for Gram-negative bacteria and to vancomycin for Gram-positive bacteria. Extended spectrum beta-lactamases were detected using the double disk diffusion test. From November 2014, carbapenemase production was detected using the modified Carbatest [21], whereas the β-CARBA test (BioRad, Marnes-la-Coquette, France) was introduced in December 2016.

Data collection

Data were collected from our SIRweb™ data concentrator (i2A, Montpellier, France) connected to our AST reading systems (Sirscan 2000 systems). The latter can read and interpret AST based on the expert system recommendations of EUCAST [22] before transfer to the laboratory information system (LIS) for biological validation in our hospital. These data were extracted and processed in a Microsoft Excel database after removing duplicates per patient to build a complete database. The number of AST performed per type of samples

Table 1 Presentation of the standard panel of antibiotics tested for the 15 most frequently isolated bacteria with the definition of wild-type strains

Strains		Antibiotics tested for the standard panel[a]											
Gram-negative bacteria													
E. coli	Wild-type	AMX	AMC	CRO	FEP	ERT	IPM	AN	GEN	CIP	SXT	FT	
		S	S	S	S	S	S	S	S	S	S	S	
P. aeruginosa	Wild-type	TIC	TCC	TZP	CAZ	FEP	IPM	AN	GEN	TOB	CIP		
		S	S	S	S	S	S	S	S	S	S		
K. pneumoniae	Wild-type	TZP	CRO	FEP	ERT	IPM	ATM	AN	GEN	CIP	SXT	FT	
		S	S	S	S	S	S	S	S	S	S	S	
E. cloacae	Wild-type	TZP	CRO	FEP	ERT	IPM	ATM	AN	GEN	CIP	SXT	FT	
		S	S	S	S	S	S	S	S	S	S	S	
P. mirabilis	Wild-type	AMX	AMC	CRO	FEP	ERT	IPM	AN	GEN	CIP	SXT		
		S	S	S	S	S	S	S	S	S	S		
K. oxytoca	Wild-type	TZP	CRO	FEP	ERT	IPM	ATM	AN	GEN	CIP	SXT	FT	
		S	S	S	S	S	S	S	S	S	S	S	
E. aerogenes	Wild-type	TZP	CRO	FEP	ERT	IPM	ATM	AN	GEN	CIP	SXT	FT	
		S	S	S	S	S	S	S	S	S	S	S	
S. marcescens	Wild-type	TCC	TZP	CRO	FEP	ERT	IPM	ATM	AN	GEN	CIP	SXT	FT
		S	S	S	S	S	S	S	S	S	S	S	S
M. morganii	Wild-type	TCC	TZP	CRO	FEP	ERT	IPM	ATM	AN	GEN	CIP	SXT	
		S	S	S	S	S	S	S	S	S	S	S	
A. baumannii	Wild-type	TIC	TCC	TZP	CAZ	IPM	AN	GEN	TOB	CIP	SXT	CS	RIF
		S	S	S	S	S	S	S	S	S	S	S	S
Gram-positive bacteria													
S. aureus	Wild-type	OXA	FOX	LZD	VA	TEC	GEN	SXT	RA	CIP	PT	CLI	FA
		S	S	S	S	S	S	S	S	S	S	S	S
S. epidermidis	Wild-type	OXA	FOX	LZD	VA	TEC	GEN	SXT	RA	CIP	PT	CLI	FA
		S	S	S	S	S	S	S	S	S	S	S	S
E. faecalis	Wild-type	AMX	VA	TEC	GEN								
		S	S	S	S								
E. faecium	Wild-type	AMX	VA	TEC	GEN								
		S	S	S	S								
S. agalactiae	Wild-type	OXA	AMX	CRO	VA	TEC	GEN	E	CLI				
		S	S	S	S	S	S	S	S				

[a] OXA, oxacillin; AMX, amoxicillin; TIC, ticarcillin; AMC, amoxicillin-clavulanate; TCC, ticarcillin-clavulanate; TZP, piperacillin-tazobactam; FOX, cefoxitin; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; ERT, ertapenem; IPM, imipenem; ATP, aztreonam; AN, amikacin; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; CS, colistin; RIF, rifampicin; FT, nitrofurantoin; LZD, linezolid; VA, vancomycin; TEC, teicoplanin; RA, rifampicin; PT, pristinamycin; E, erythromycin; CLI, clindamycin; FA, fusidic acid

was analysed, and a specific file was extracted for each of the 15 most commonly isolated bacteria to determine their distribution by sample type.

Resistance analysis

Each antibiotic tested is categorised as "susceptible" or "resistant" to simplify the analysis, and thus, intermediate strains were considered as likely resistant.

For each bacterial species, the overall percentage of resistance for each antibiotic tested per year and per month was calculated to obtain a complete overview. A specific database was build including only isolates from blood cultures, which were considered invasive infections for analysis.

We then specifically assessed the resistance rate to agents used as empirical treatment as defined in Table 2. Therefore, ceftriaxone and carbapenems (i.e. imipenem) were selected for Gram-negative bacteria, whereas cefoxitin, vancomycin, and cotrimoxazole were selected for *Staphylococcus* species. Similarly, the resistance rate to amoxicillin, vancomycin, and ceftriaxone was evaluated for *Enterococcus* and *Streptococcus* species.

Finally, a specific analysis was conducted for bacteria, which included the top 15, to evaluate the evolution of non-resistant

strains over these 3 years. A non-resistant strains strain was defined as having no acquired resistance to the panel of antibiotics tested presented in Table 1.

Classification of resistance

In this section, we focused only on five bacterial species corresponding to the most critical indicators by the Infection Control Committee, including *E. coli*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *S. aureus*. To complete our analysis, we classified the resistance to key antibiotics by functional categories for all strains tested during the 3-year period. We considered key antibiotics those used as empirical treatments, including third-generation cephalosporins and imipenem for Gram-negative bacteria, and methicillin and vancomycin for *S. aureus*. The list of our definitions, including MDR, XDR (extensively drug-resistant), and PDR (pan-drug resistant), are presented in Table 2. To classify our strains, if resistance to the complete panel of antibiotics tested was obtained, we analysed a second panel of antibiotics corresponding to the second line of antibiotics tested, including fosfomycin, colistin (MICs), minocycline, and tigecycline.

Data comparison with our historical blood cultures database

In a second analysis, the local results of resistance for invasive infections, defined as positive blood cultures, were compared to historical bacteraemia database results obtained from 2001 to 2014 from our hospital with key antibiotics (ceftriaxone for *E. coli* and *K. pneumoniae*, imipenem for *P. aeruginosa* and *A. baumannii* and methicillin for *S. aureus*).

Statistical analysis

The data were analysed by a chi-squared test. Significance was assessed at $p < 0.05$.

Results

Data collection

Over the 3-year study period, 99,932 ASTs were performed in our hospital to build our database. We obtained a total of 229 different bacterial species, presented in Table S2, for the 3-year-period. The three most common samples were urine (40.7%; $n = 40{,}624$), blood cultures (13.0%; $n = 13{,}806$), and respiratory specimens (9.4%; $n = 9416$) (Fig. 1).

The 15 most frequent bacteria isolated in our hospital, which represent 82.9% of all bacterial isolates ($n = 82{,}829$), were, by order of frequency, *E. coli* ($n = 27.240$; 27.3%), *S. aureus* ($n = 14{,}052$; 14.1%), *P. aeruginosa* ($n = 8626$; 8.6%), *S. epidermidis* ($n = 7660$; 7.7%), *K. pneumoniae* ($n = 6260$; 6.3%), *E. faecalis* ($n = 5560$; 5.6%), *E. cloacae* ($n = 3700$; 3.7%), *P. mirabilis* ($n = 2672$; 2.7%), *S. agalactiae* ($n = 1797$; 1.8%), *K. oxytoca* ($n = 1100$; 1.1%), *E. aerogenes* ($n = 1096$; 1.1%), *E. faecium* ($n = 1033$; 1.0%), *S. marcescens* ($n = 858$; 0.9%), *M. morganii* ($n = 853$; 0.9%), and *A. baumannii* ($n = 322$; 0.3%) (Fig. 2). The distribution of the top 15 was dependent on the nature of samples, with *S. epidermidis* predominant ($n = 3045$; 23.4%) from blood cultures, whereas *S. aureus* ($n = 3227$; 33.9%) and *P. aeruginosa* ($n = 2049$; 25.3%) were the most frequent bacterial species isolated from respiratory samples. The number of urine samples represents 40.7% of all samples, for which *E. coli* is the most frequently detected bacterial species ($n = 19{,}808$; 48.6%) (Fig. 3).

Resistance analysis

All isolates

The rate of non-resistant strains among Gram-negative bacteria included in the top 15 increased in 2016 compared to 2014 and 2015, with a significant difference for *E. coli* (X^2; $p = 0.0004$),

Table 2 Definition of the different classifications of resistance by functional categories

Type of strains	Definitions
Wild-type strains	No acquired resistant; susceptible to a classic panel of antibiotic presented in Table 2
MDR (multi-drug resistant)	Resistance to all empirical treatment: - Gram-negative bacteria[a]: third-generation cephalosporins and imipenem - Gram-positive bacteria: *S. aureus*: it included methicillin resistance and for *Enterococcus*: it included resistance high level of resistance to penicillin and resistance to vancomycin.
XDR (extensively drug-resistant)	Resistance to all antibiotics except for two classes of antibiotics
PDR (pan-drug resistant)	Resistance to all classes of antibiotics

[a] Empirical treatment for *Enterobacteriaceae* and non-fermentative Gram-negative bacteria included third-generation cephalosporins (ceftriaxone for *E. coli* and *K. pneumoniae* and ceftazidime for *A. baumannii* and *P. aeruginosa*)

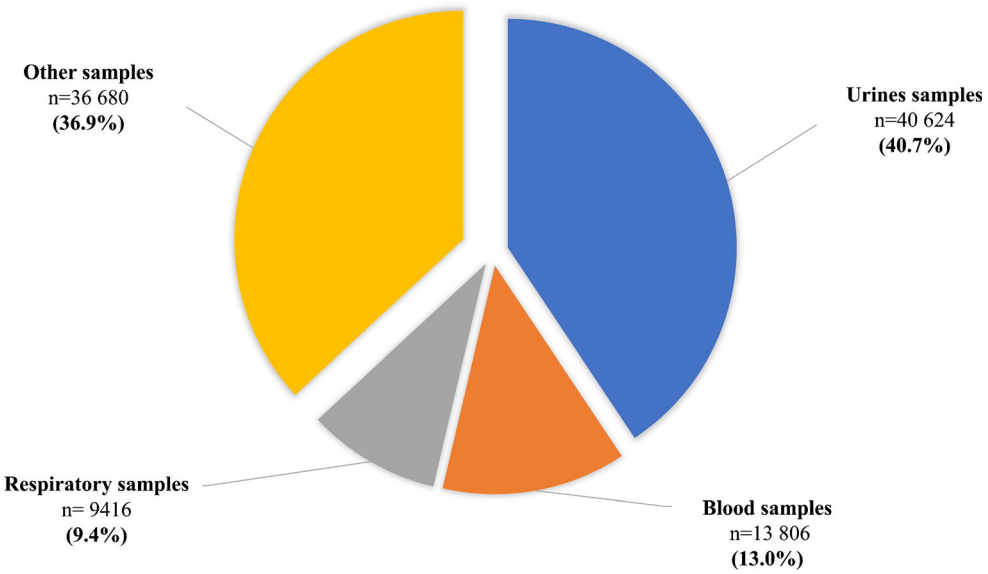


Fig. 1 Distribution by sample type over the 3-year period for the 99,932 ASTs

P. mirabilis (X^2; $p = 0.04$), and *S. marcescens* (X^2; $p = 0.04$). The same rate decreased only for *M. morganii* and *P. aeruginosa* (Fig. 4). For *E. coli*, the percentage of non-resistant strains in 2014 was 35.6% compared to 39.8% in 2016 (p value = 0.0004). In 2016, the percentage of non-resistant strains represent more than 50% of all ASTs performed for *S. marcescens* (88.5%), *Enterobacter* species, (50.8%) and *P. mirabilis* (52.6%). The percentages of resistance for all antibiotics tested per year for the top 15 bacteria are presented in Tables S3 and S4, with a specific table for positive blood cultures compared to other samples.

For *Enterobacteriaceae* and key antibiotics (i.e. ceftriaxone and imipenem), the level of ceftriaxone resistance for *K. pneumoniae* strains, *Enterobacter* species (*E. cloacae* and *E. aerogenes*), and *M. morganii* was not significantly different during the study period (Fig. 4). An increase of imipenem resistance was noted for *M. morganii*, with a prevalence of more than 20% in 2016, but without carbapenemase production. For *K. pneumoniae*, we observed a small increase, with 1.5% in 2014 and 3.4% in 2016 ($p = 0{,}001$). For the other species, the main level of resistance to imipenem during the 3-year period was 3.2% in blood cultures and < 5% in other samples for Gram-negative bacteria in the top 15, including non-fermentative bacteria (*P. aeruginosa* and *A. baumannii*).

For Gram-positive bacteria, non-resistant strains increased with a significant difference for *S. aureus* (X^2; $p = 0.03$) and

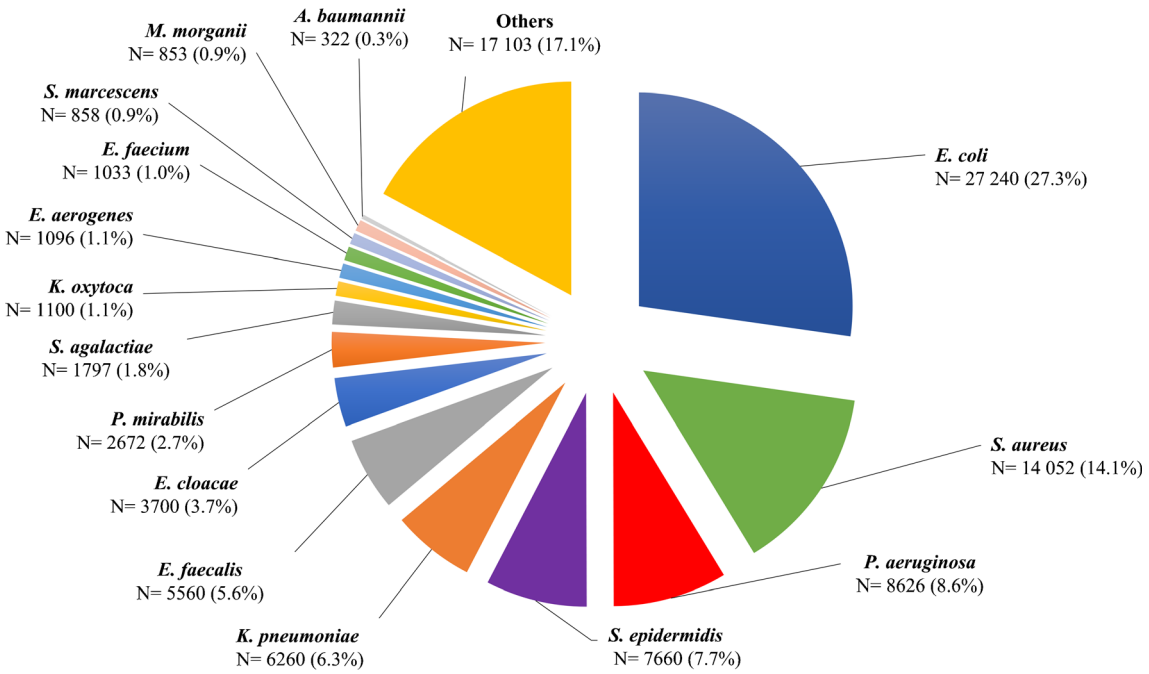


Fig. 2 Presentation of the 15 most frequently isolated bacteria in Marseille Hospital Center between January 2014 and November 2016

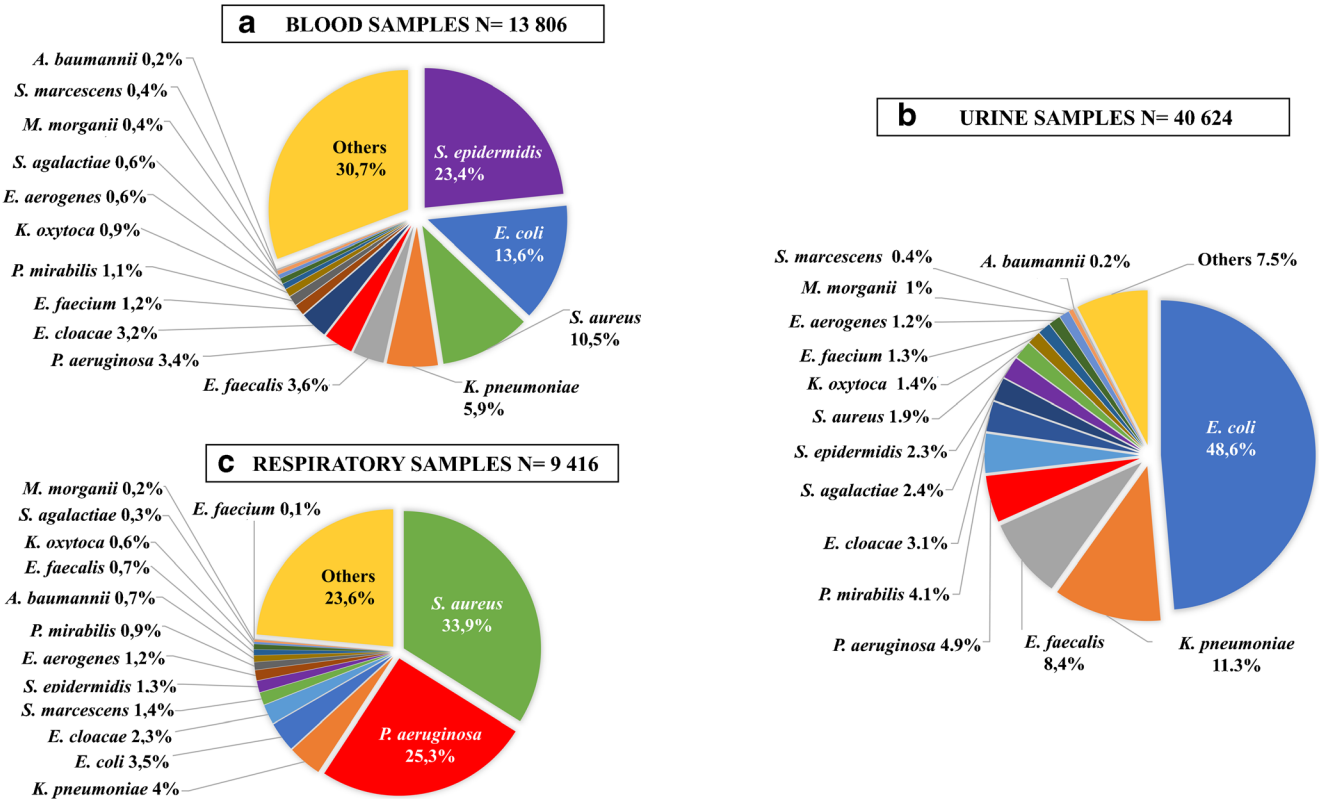


Fig. 3 Distribution of the 15 most frequently isolated bacteria in La Timone hospital from different samples between January 2014 and November 2016. **a** Blood samples, **b** urine samples, and **c** respiratory samples

S. agalactiae (X^2; $p = 0.02$) (Fig. 5). The level of resistance to vancomycin was very low < 1.4% for all Gram-positive bacteria. For *S. aureus*, the percentage of methicillin resistant *S. aureus* (MRSA) strains did not significantly evolve during the 3 year period, ranging from 14.3 to 12.8%.

Isolates from blood cultures

For all bacteria isolated from positive blood cultures (n = 13,806), the percentage of non-resistant strains increased significantly from 2014 to 2016, with 30.6 and 35.5%, respectively (Figs. 6 and 7).

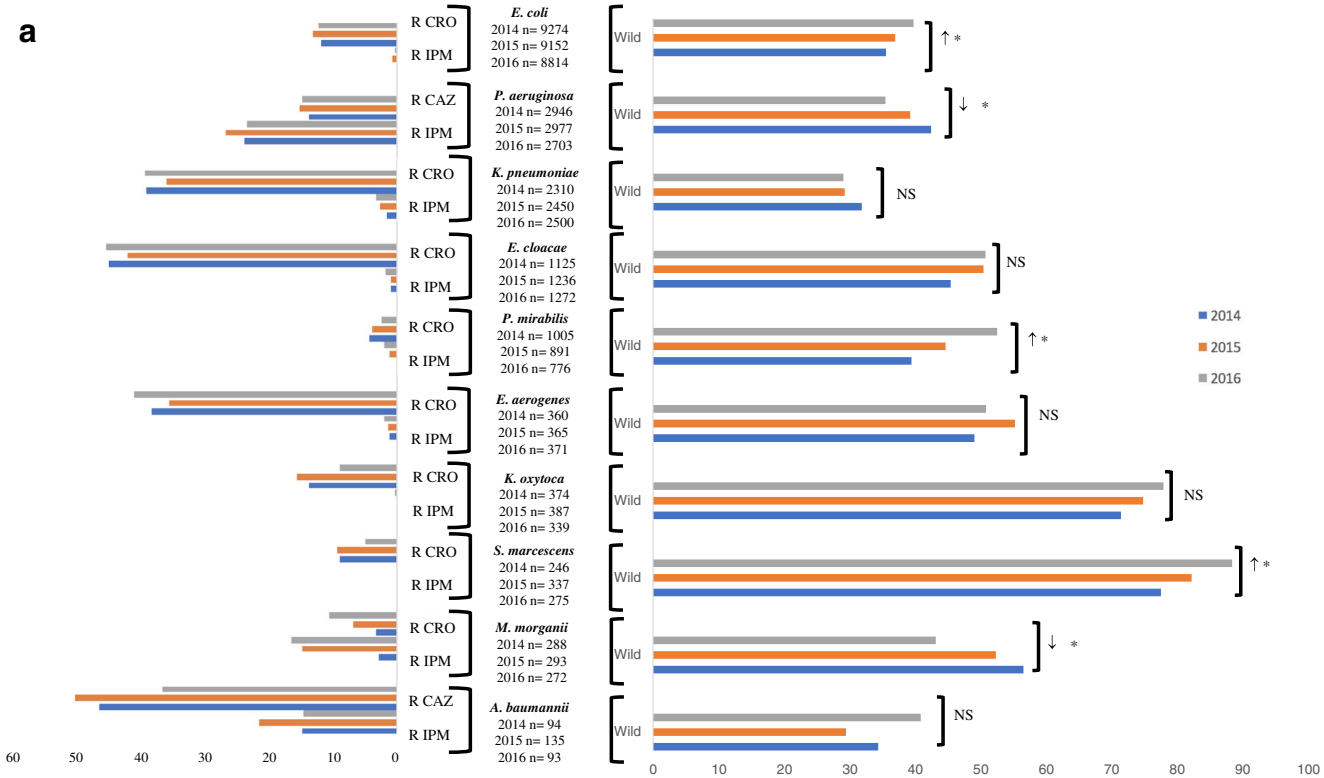
In the top 15 bacteria, the strains isolated from positive blood cultures were likely more resistant than those isolated from other samples, with a significant difference for *E. coli*, *S. aureus*, and *S. epidermidis* (Tables S3 and S4). Indeed, the percentage of resistance for penicillins, penicillins + inhibitors, and cephalosporins was higher in blood cultures compared to other samples for *E. coli* ($p < 10^{-5}$). For *S. aureus*, the percentage of MRSA in 2016 in blood samples was 18.9% compared to 11.7% for other samples ($p = 0.0002$). The same trend was observed for *S. epidermidis*, with a methicillin-resistance rate of 77.1 and 60.4% in positive blood cultures and other specimens, respectively ($p = 0.00009$).

Classification of resistance

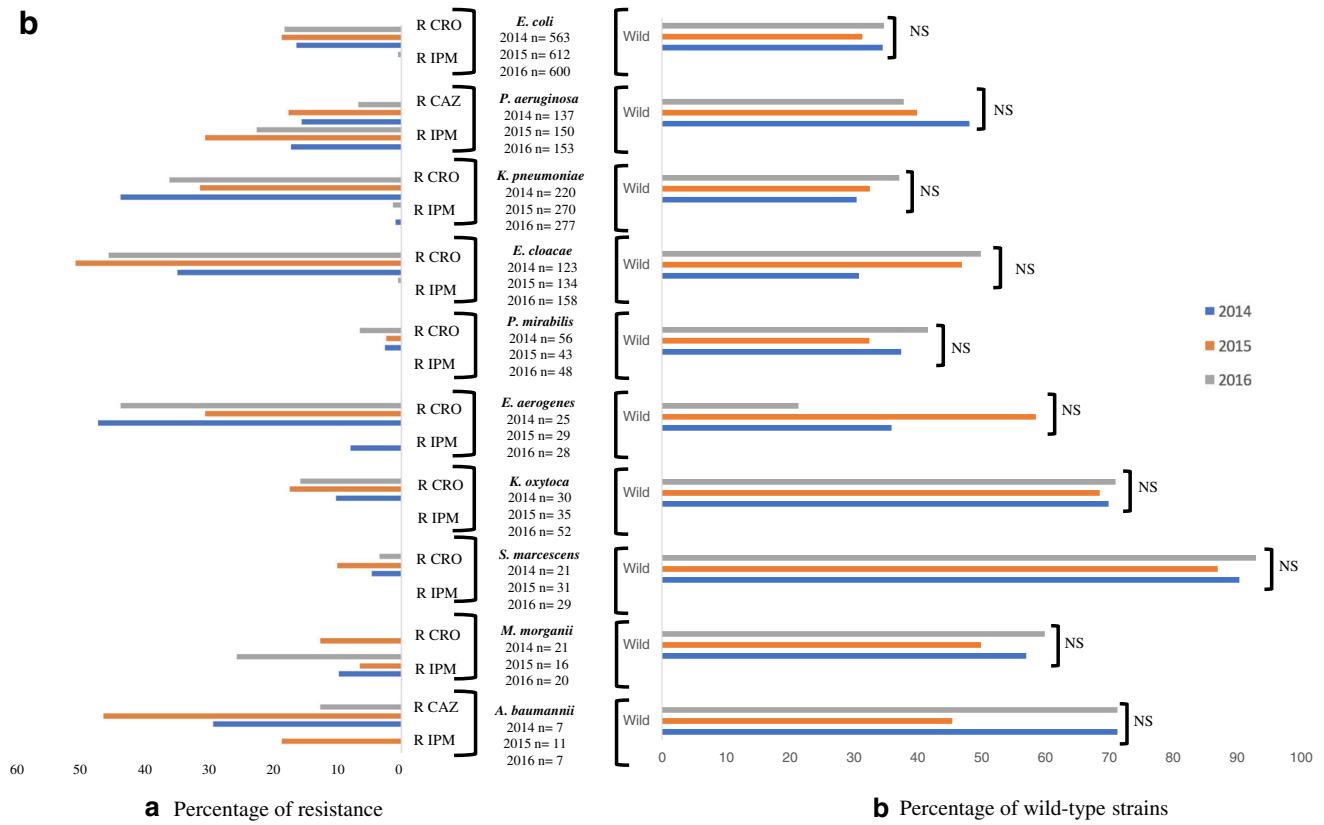
Among the 27,187 *E. coli* strains, 38.1% (n = 10,356) displayed a non-resistant phenotype (i.e. fully susceptible to the panel of antibiotics tested) with 0.6% MDR- (n = 163), 0.03% XDR- (n = 8), and 0%-PDR strains (Table 3). MDR and XDR strains correspond to carbapenemase-producing *E. coli*. For *K. pneumoniae*, the percentage of non-resistant strains was 33.9%, whereas that of XDR and PDR strains was 2.3 (n = 173/7253) and 0.1% (n = 7/7253), respectively. These strains were mostly $bla_{\text{OXA-48}}$ producers. For *P. aeruginosa* and *A. baumannii*, 40% were non-resistant strains (n = 3449, 40.4% for *P. aeruginosa* and n = 111, 40.1% for *A. baumannii*). The XDR-rate was 2.2% for *P. aeruginosa* (n = 188) and 14.4% for *A. baumannii* (n = 40), respectively. The overall rate of XDR and PDR was 0.5% (n = 409/ 82,829) and < 0.03% (n = 11/43260), respectively. For six strains, there is a lack of information because only the standard panel was tested.

Data comparisons with our historical blood cultures database

Comparison with our historical blood culture database over 16 years (2001–2016) in Marseille highlights an increase of resistance to third-generation cephalosporins for *E. coli*



a Percentage of resistance **b** Percentage of wild-type strains



a Percentage of resistance **b** Percentage of wild-type strains

Fig. 4 Presentation of the evolution over 3 years for the most frequent Gram-negative bacteria. **a** Percentage of resistance to specific antibiotics, **b** percentage of strains with a wild type profile. **a** For all samples, **b** for positive blood cultures. R CRO, resistance to ceftriaxone; R IPM, resistance to imipenem; R CAZ, resistance to ceftazidime.

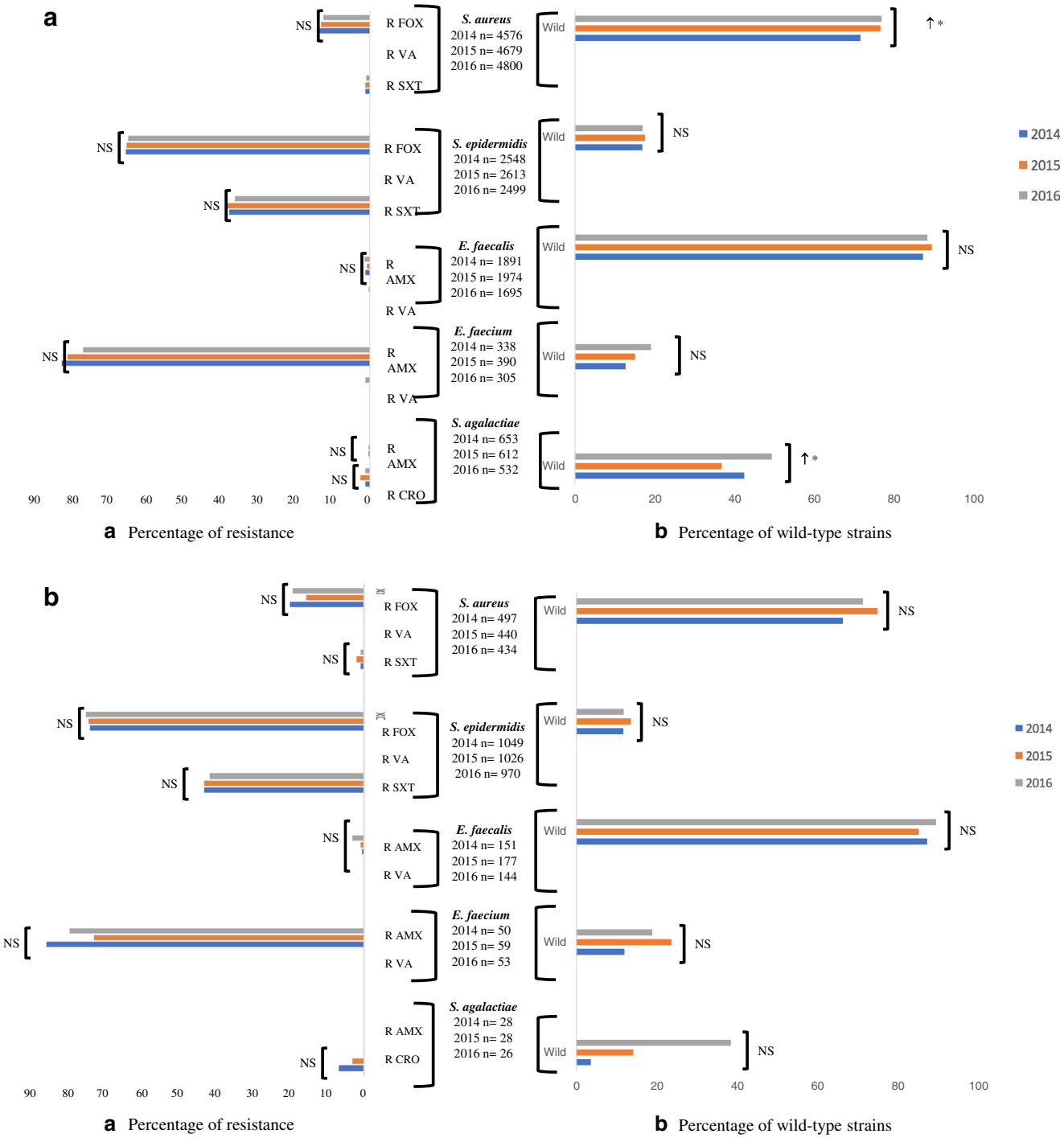


Fig. 5 Presentation of changes over 3 years for the most frequent Gram-positive bacteria. **a** Percentage of resistance of specific antibiotics, **b** percentage of strains with a wild type profile. **a** For all samples, **b** for positive blood cultures. R FOX, resistance to cefoxitin; R AMX, resistance to amoxicillin; R VA, resistance to vancomycin; R SXT, resistance to trimethoprim-sulfamethoxazole; R CRO, resistance to ceftriaxone

(2001 = 0% vs 2016 = 17.8%; $p < 10^{-5}$) and *K. pneumoniae* (2001 = 8% vs 2016 = 35.4%; $p = 0.001$) (Fig. 8) along with a d`ecrease of MRSA strains (2001 = 31% vs 2016 = 19.8%; $p = 0.006$). For *A. baumannii*, we observed some sporadic outbreaks during 2 years (2012 and 2015), without an increase of the resistance to imipenem (2016 = 0% resistance to imipenem). For *P. aeruginosa*, the level of resistance to imipenem increased and decreased during the 16-year period with a mean of 23%. In 2001, the level was 29.8%, and in 2016, it was 22.1%. This resistance seems to be balanced over time with variations as a function of years.

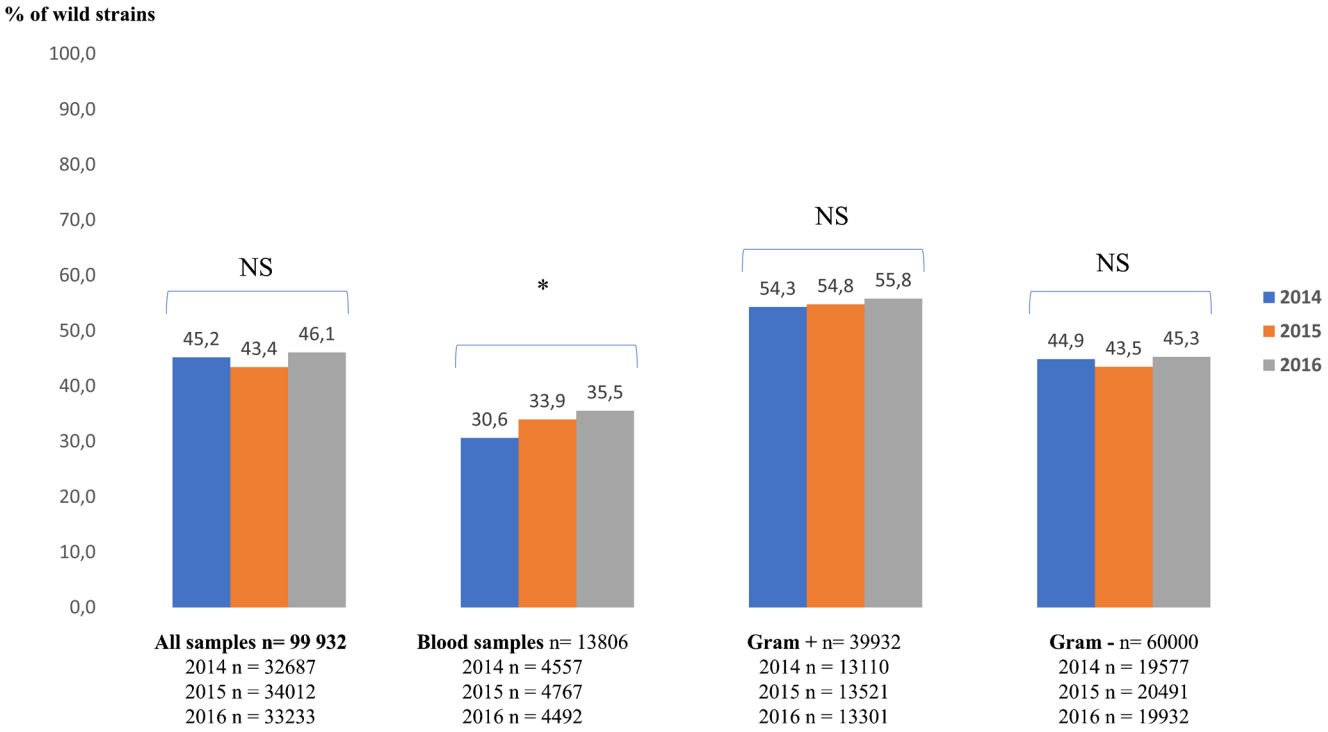


Fig. 6 Presentation of the percentage of wild-type strains in different samples

Discussion

Following the spread of alarmist speculation about the increase of MDR bacteria, we tried in objectively analyse overall AST data (≈ 100 00 ASTs) in our hospital centre. Here, we provide an overview of the local epidemiology of antibiotic resistance based on the 15 most frequently isolated bacteria in Marseille hospitals after verification.

Our results show a significantly higher prevalence of susceptible strains in 2016 compared to 2014 (non-resistant strains) for *E. coli*, *P. mirabilis*, *S. marcescens*, *S. aureus*, and *S. agalactiae*. In our city, we found a low level of resistance to carbapenem in all Gram-negative bacteria of 5% for all samples. The same level falls to 3.2% when blood cultures only are considered. In addition, we did not identify vancomycin-resistance in *S. aureus*, whereas the MRSA rate decreased beginning in 2010. Regarding blood culture, empirical treatment is effective for 99.4% of *Enterobacteriaceae* of the top 15 bacteria, corresponding to susceptibility to imipenem and for 100% of cases in *S. aureus*. Moreover, resistance to antibiotics in blood cultures tends to balance with species that are more resistant (*K. pneumoniae* with resistance to third-generation cephalosporins) and species that become less resistant (MRSA).

The problem of MDR bacterial infection does not concern the problem of therapeutics, but to an adapted empirical treatment to prevent the death of patients [23]. This

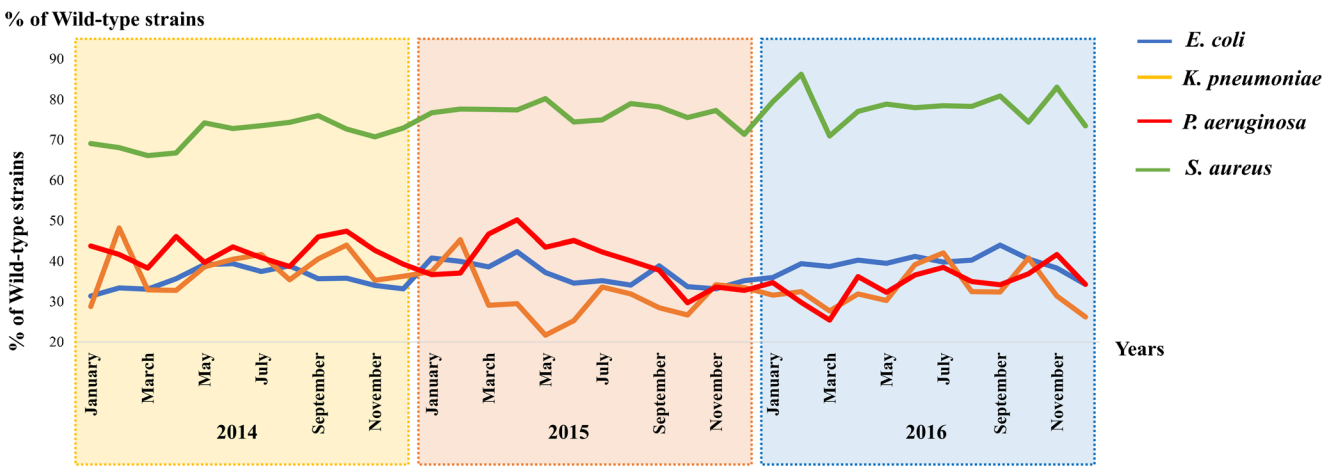


Fig. 7 Presentation of the percentage of wild-type strains in all samples between 2014 and 2016

Table 3 Classification of resistance per functional categories for all strains isolated during 2014 to 2016 for *E. coli*, *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*

Bacterial species	Classes of antibiotics	Number of strains	%
E. coli	Wild-type	10,356	38.1%
	R to third-generation cephalosporins (CRO[d])	3242	11.9%
	MDR[a]	163	0.6%
	XDR[b]	8	0.03%
	PDR[c]	0	0%
	Total	27,187	100%
K. pneumoniae	Wild-type	2462	33.9%
	R to third-generation cephalosporins (CRO)	2679	36.9%
	MDR	169	2.3%
	XDR	173	2.4%
	PDR	7	0.1%
	Total	7253	100%
P. aeruginosa	Wild-type	3449	40.4%
	R to third-generation cephalosporins (CAZ[e])	1272	14.9%
	MDR	810	9.5%
	XDR	188	2.2%
	PDR	4	0.05%
	Total	8543	100%
A. baumannii	Wild-type	111	40.1%
	R to third-generation cephalosporins (CAZ)	123	44.4%
	MDR	49	17.7%
	XDR	40	14.4%
	PDR	0	0%
	Total	277	100%

[a] MDR (multi-drug resistant)

[b] XDR (extensively drug-resistant)

[c] PDR (pan-drug resistant)

[d] CRO: ceftriaxone

[e] CAZ: ceftazidime

requires the use of surveillance systems to monitor the resistance rate of keys antibiotics used as empirical treatment to prevent a potential spread of antimicrobial resistance [24]. This is emphasised by the high susceptibility rate of isolates recovered from blood cultures to first-line antimicrobial agents (> 99% of strains) found in the present study. Thus, the level of carbapenem resistance for *Enterobacteriaceae* in Marseille is in fact not significant, although this city has attracted various migratory movements. However, we found that the resistance to carbapenems increases from 1.5% in 2014 to 3.4% in 2016 for *K. pneumoniae*. But in fact, the rate of carbapenemase-production remains stable as it was of 0.69 and 0.76% in 2014, 2015, and 2016, respectively. Moreover, more than the half of these strains was considered as carriage (from rectal, cutaneous, pharyngeal, or urine specimen). It has also not be noticed that the number of specimen received and dedicated to the screening of antimicrobial resistance is continuously increasing. These data do not support a current spreading of carbapenemase encoding genes from bacteria isolated among our hospitals. Carbapenem administration in first intention in case of sepsis involving *Enterobacteriaceae* thus seems reasonable considering a possible de-escalation since AST is available. Impact on ecology of such strategy has to be nevertheless further evaluated, but we have previously shown that alternatives exist mainly belonging to the "forgotten antibiotics" even allowing to break the beta-lactam cycle [25]. In parallel, the massive use of 3GC could be reconsidered as yielded by the remarkable efficacy of pivmecillinam in uncomplicated UTI involving ESBL-producing *Enterobacteriaceae* [26].

Rather than deliberating how to face a hypothetical disaster, we should ensure that empirical therapy is effective therapy. Detecting MDR bacteria in clinical microbiology laboratories within 24 h is the first challenging step. Different techniques were recently developed, including the rapid detection of resistance genes by phenotypic or molecular tests [27, 28],

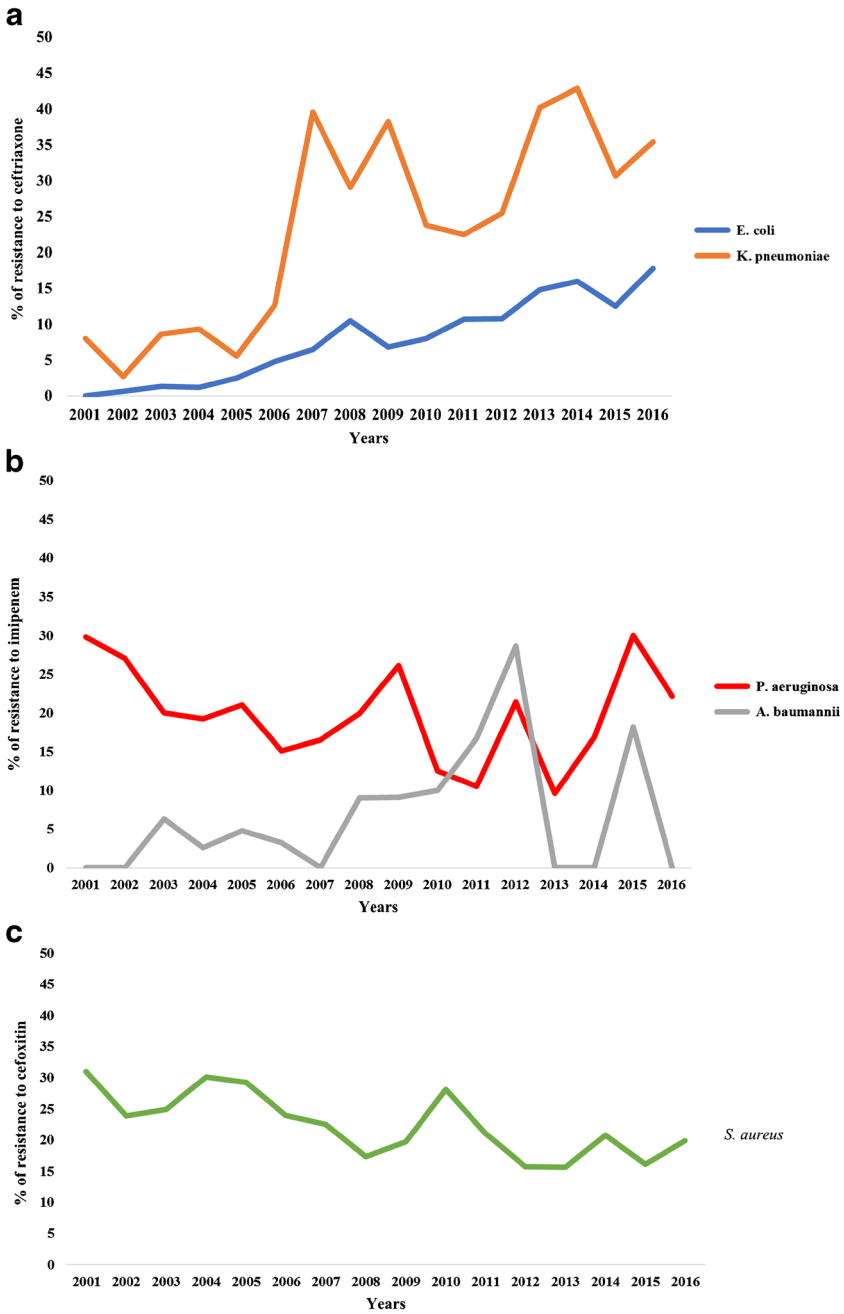


Fig. 8 Main multi-resistance markers in positive blood cultures between 2001 and 2016

the use of real-time video imaging [29], and the microbiology laboratory, which must be reactive by proposing alternative antibiotics when resistance to empirical treatment is detected (Fig. 9). Moreover, these drugs must be available [30]. Upstream, understanding of the local epidemiology is necessary to develop guidelines to adapt empirical treatments to the local ecology. It remains crucial to monitor the various levels of resistance of key antibiotics using specific surveillance for some antibiotics, such as third-generation cephalosporins, carbapenems, vancomycin, and colistin. The detection of abnormal events using these tools enables the adaptation of empiric therapeutic strategies.

We are nevertheless aware of several limitations of this study. First, as a limited number of antibiotics was tested in first intention, some resistance mechanisms could have been missed in particular concerning aminoglycosides and *Enterobacteriaceae*. We also assume that our definition of MDR-bacteria does not depend of the number of antibiotics classes for which the antibiotic is resistant. This classical definition does not take account of the number of antibiotics tested and unfairly categorises as resistant any class for which a molecule is inactive irrespective to other agents belonging to the same class [14]. Finally, if this study was conducted at a local scale and cannot be generalised, our data underline that

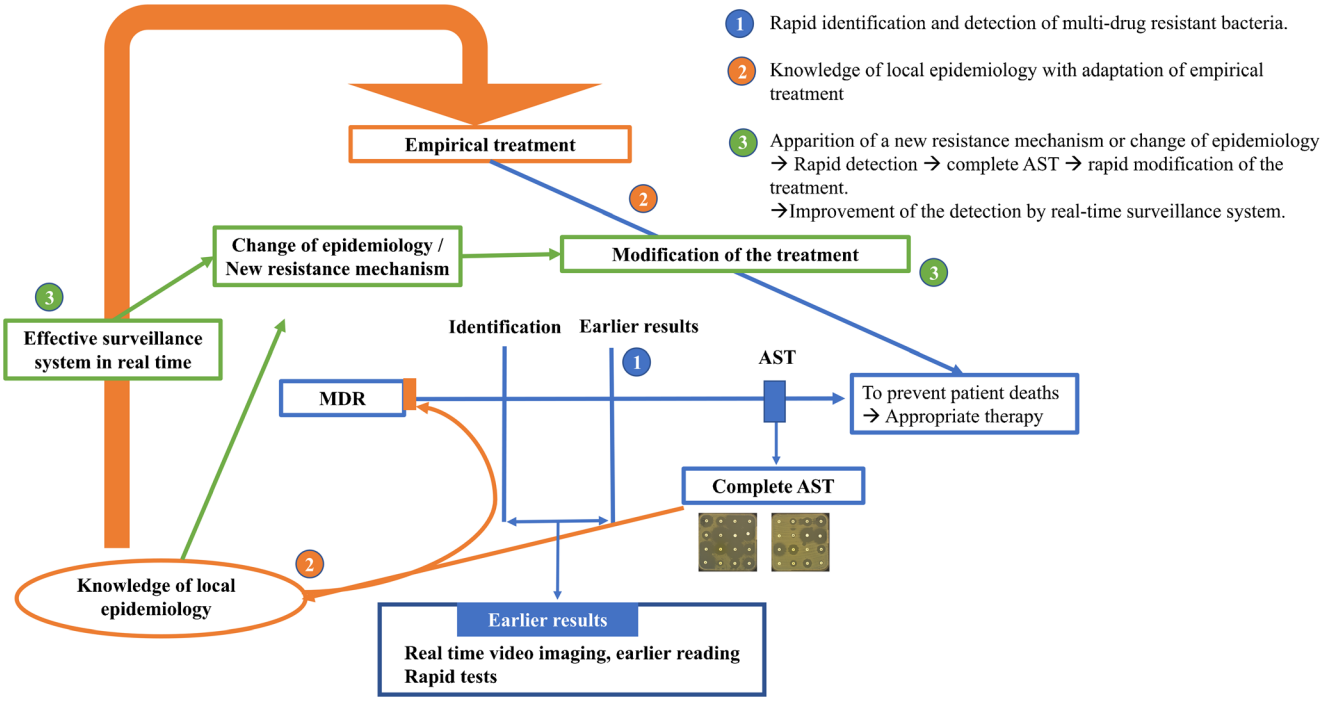


Fig. 9 How can an empirical treatment be modified based on local epidemiology?

knowledge about local epidemiology enable adaptation of guidelines for empirical treatment.

In summary, antibiotics are part of a complex ecosystem, in which types of resistance and tolerance to different microorganisms are highly heterogeneous. Resistance to antibiotics existed long before the discovery of antibiotics. Among other things, bacteria have acquired the ability to remove exogenous genes, using CRISPR/anti-CRISPR systems [31]. The selective pressure of the different ecosystems studied showed that bacteria differed according to their origin. Hospital bacteria are different from the bacteria originating from the environment. Moreover, selective pressures diffuse bacterial clones; the presence and disappearance of which are poorly known. This is notable for strains of *S. aureus* ST30 and ST31, which were endemic and have recently decreased without known explanation [13]. However, the evolution of resistance to antibiotics remains unpredictable [8]. In this study, the number of susceptible strains increased between 2014 and 2016, with an increase in *K. pneumoniae* producing ESBL and a decrease in MRSA strains. The observed is not a problem of resistant bacteria, but that the resistances observed are no longer the same, because we have not identified the evolution of these resistances.

Conclusions

This analysis demonstrates a global low level of resistance to key antibiotics in Marseille, France that should be surveyed in the future. The combination of real-time surveillance of predominant phenotypes of resistance to antibiotics for the most frequent bacteria, along with specific alarms implemented in automatic surveillance systems, enable the surveillance and detection of abnormal events linked to antibiotic resistance. We recently showed that pan-drug resistant bacteria are extremely rare when we test a large panel of antibiotics, including old drugs that are usually not tested in clinical microbiology laboratories. This strategy will help to better define empirical treatment based on the true prevalence of resistance at a local level (Fig. 9). Therefore, our study demonstrates that the level of resistance to antibiotics of the most common bacteria involved in infections is less than the recent alarmist publications and prediction of mortality due to MDR. We have recently underlined that human deaths due to PDR bacteria are in fact very rare even in literature [32]. Evidence-based medicine relies on real-life data rather than on hypothetical models.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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