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In vitro antimicrobial susceptibility of equine clinical isolates from France, 2006–2016

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ABSTRACT

Objectives: This study aimed to analyse antimicrobial susceptibility evolution of equine pathogens isolated from clinical samples from 2006–2016.
Methods: A collection of 25 813 bacterial isolates was studied, clustered according to their origins (respiratory tract, cutaneous, genital and other), and analysed for their antimicrobial susceptibility using the disk diffusion method.
Results: The most frequently isolated pathogens were group C *Streptococci* (27.6%), *Escherichia coli* (20.0%), *Staphylococcus aureus* (7.8%), *Pseudomonas aeruginosa* (4.0%), *Enterobacter* spp. (3.4%), *Klebsiella pneumoniae* (2.4%), and *Rhodococcus equi* (1.8%). Of the isolates, 9512 were from respiratory samples (36.8%), 7689 from genital origin (29.8%), and 4083 from cutaneous samples (15.8%). Over the 11-year period, the frequency of multidrug-resistant (MDR) strains fluctuated between 6.4–20.4% for group C *Streptococci* and 17–37.7% for *Klebsiella pneumoniae*. From 2006–2009, 24.5–43.0% of *Staphylococcus aureus* isolates were MDR; after 2009 the level did not exceeded 27.6%. For *Escherichia coli* and *Enterobacter* spp., these levels were mostly >30.0% until 2012, but significantly decreased thereafter (22.5–26.3%).
Conclusions: This study is the first large-scale analysis of equine pathogens, by the number of samples and duration of study. The results showed high levels of MDR strains and the need to support veterinary antimicrobial stewardship to encourage proper use of antibiotics.

1. Introduction

The overuse and misuse of antimicrobials in both human and veterinary medicine have contributed to the emergence and spread of multidrug-resistant (MDR, bacteria resistant to three or more antimicrobial classes) bacterial pathogens around the world. Since the end of the 20th century, this phenomenon has become a major public health problem and a priority for international institutions such as the World Health Organization (WHO, www.who.int), the Food and Agriculture Organization of the United Nations (FAO, www.fao.org) and the World Organization for Animal Health (OIE, www.oie.int), which published a list of recommendations and guidelines to counteract MDR emergence. This problem is all the more alarming since transmission of MDR pathogenic strains from animals to humans has also been observed [1,2].

In this context, several studies aiming to describe the evolution and prevalence of resistance in equine pathogens have been conducted in different countries such as South Africa [3], Canada [4,5], Switzerland [6] and the United Kingdom [7]. These studies have reported a high level of MDR among horse pathogens, which complicates therapy with commonly used antimicrobial drugs (i.e., penicillins, cephalosporins, gentamicin or cotrimoxazole). This underlines the importance of performing antimicrobial susceptibility testing, in order to optimise antimicrobial therapy in horses

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and reduce the risk of development of resistance. Moreover, because of the close relationships between horses and humans, these bacteria may act as a reservoir of MDR bacteria.

In France, a national surveillance network for antimicrobial resistance in pathogenic bacteria of animal origin (RESAPATH, www.resapath.anses.fr), which has been in place for cattle since 1982 and was extended to horses in 2007, provides annual data about antimicrobial susceptibility. Moreover, a French national plan (ECOANTIBIO) was conducted during 2012–2016 with the objectives of reducing exposure of animals to veterinary antibiotics by 25.0% and then to preserve a therapeutic arsenal of antibiotics in a durable way. The recently collected data showed a 37.0% decrease in animal exposure to antibiotics over the period 2012–2016 (http://agriculture.gouv.fr/ecoantibio). The decline was even more marked for critical antibiotics (–75.0% for fluoroquinolones and –81.0% for last-generation cephalosporins), and has resulted in regulatory restrictions for their prescription since 2016. Following this positive dynamic, the ECOANTIBIO 2 Plan was set up for the period 2017–2021 (http://agriculture.gouv.fr/le-plan-ecoantibio-2-2017-2021).

Nevertheless, data on MDR strains isolated from horses in France are rarely available. The objective of this study was to describe the antimicrobial susceptibility profiles of equine pathogens over an 11-year period (2006–2016). For this purpose, >25 000 isolates recovered from different infection sources from horses in France were collected and phenotypically analysed.

2. Materials and methods

2.1. Bacterial isolates

From January 2006 to December 2016, 25 813 bacterial isolates were retrospectively included in the study (2006: n = 1582; 2007: n = 1923; 2008: n = 2281; 2009: n = 2392; 2010: n = 2605; 2011: n = 2915; 2012: n = 3058; 2013: n = 2477; 2014: n = 2373; 2015: n = 2312; 2016: n = 1895). These isolates were issued from samples of horses collected by numerous practitioners all over France. They were all recovered from horses with a suspected bacterial infection (with no prior antimicrobial treatment) and classified into four groups according to the origin of infection: respiratory (including nasal, nasopharyngeal, tracheal, bronchial and guttural pouch washes, swabs, secretions or aspirations), cutaneous, genital, and others. All analyses were carried out in the current veterinary microbiology diagnostic unit.

2.2. Phenotypic identification

To isolate and identify strains, non-selective (Columbia agar with 5% sheep blood) and selective media (Columbia CNA agar with 5% sheep blood and Eosin methylene blue agar) were used. When more than three different species were isolated in a sample, it was considered as 'contaminated' and no further analysis was performed. Isolates were definitively identified by Gram staining, followed by either standard biochemical tests or commercially available identification systems, such as API and VITEK 2 Compact® systems (bioMérieux, Marcy l'Étoile, France), or MALDI-TOF mass spectrometry technology (Microflex; Bruker Daltonics, Bremen, Germany) according to the manufacturers' instructions.

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) was performed using the disc diffusion method on Mueller-Hinton agar (enriched with 5.0% sheep blood for *Streptococcus* spp.) according to the recommendations of the European Committee on Antimicrobial

A

Identified microorganisms	*Total 2006-2016* *25813 (100%)*
Streptococcus (group C)	7124 (27.6%)
Escherichia coli	5159 (20.0%)
Staphylococcus aureus	2012 (7.8%)
Pseudomonas aeruginosa	1034 (4.0%)
Enterobacter spp	878 (3.4%)
Klebsiella pneumoniae	626 (2.4%)
Rhodococcus equi	462 (1.8%)
Other bacteria	8518 (33.0%)

B

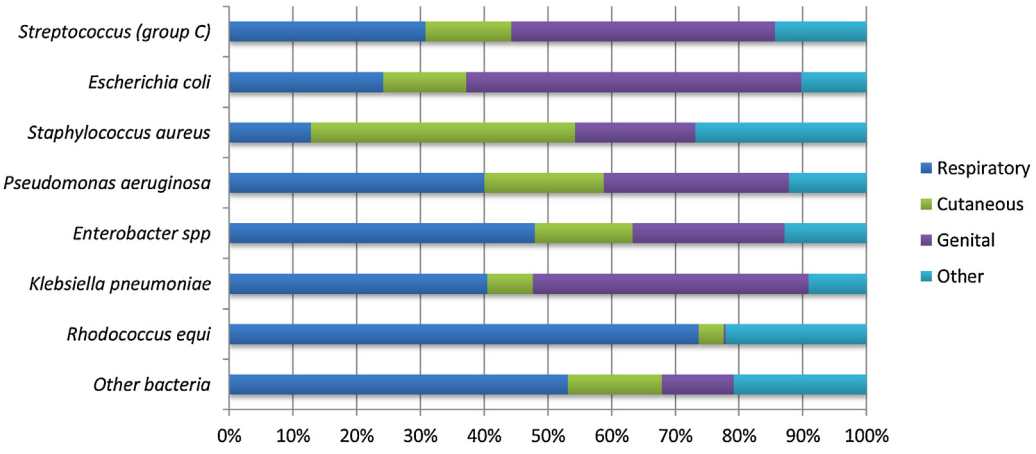

Fig. 1. Distribution of 25 813 antimicrobial susceptibility tests (2006–2016) in relation with the principal identified microorganisms (A) and sample group (B).

Table 1
Percentage of resistant bacteria per year. A, B and C. Percentage of resistant bacteria for relevant Gram-positive species. D, E, F and G. Percentage of resistant bacteria for relevant Gram-negative species.

A		% of resistant *Streptococcus* (group C)										
Antibiotic category		**Year**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**
		Number of strains (n)	**383**	**464**	**554**	**627**	**671**	**703**	**811**	**680**	**768**	**771**	**692**
Penicillins		PEN	0.0	0.4	0.2	0.0	0.0	0.1	0.1	0.0	0.1	0.0	0.1
		AMP/AMOX	0.0	0.2	0.2	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.7
		OXA	NT	NT	NT	NT	NT	NT	0.1	0.0	0.1	0.0	0.1
		AMC	0.0	0.2	0.2	0.0	0.0	0.1	0.2	0.0	0.0	0.0	0.7
Cephalosporins	**3rd**	CEF	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4
	4th	CEQ	0.0	0.0	0.2	0.0	0.0	0.0	0.2	0.1	0.0	0.0	0.1
Aminoglycosides		STR^{HC}	7.0	7.3	6.1	5.9	7.0	6.4	4.9	5.9	3.4	6.2	5.5
		KAN^{HC}	NT	NT	NT	NT	NT	NT	4.7	5.3	3.6	6.9	5.3
		GEN^{HC}	1.0	2.4	1.6	0.8	2.7	2.7	2.3	1.8	1.2	0.8	0.6
Tetracyclines		TET/OT**	22.2	33.4 *	33.8	34.0	25.8 *	44.1 *	45.3	75.7 *	61.5 *	63.9	82.1*
Macrolides		ERY	8.6	14.4 *	5.6*	13.2 *	6.7*	9.1*	6.0	6.5	6.6	8.8	11.1
Rifampicin		RIF**	0.5	1.1	0.5	0.6	0.4	1.0	0.1*	0.0	0.3	0.4	15.5*
Sulphonamides		SUL/SXT**	1.0	6.0*	14.8 *	1.4*	0.6	1.6	23.1 *	0.1*	1.0*	0.0*	4.8*

B		% of resistant *Rhodococcus equi*										
Antibiotic category		**Year**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**
		Number of strains (n)	**22**	**36**	**50**	**42**	**61**	**70**	**53**	**43**	**45**	**33**	**7**
Macrolides		ERY**	13.6	0.0*	0.0	4.8	1.6	1.4	1.9	0.0	0.0	0.0	0.0
Rifampicin		RIF	0.0	0.0	2.0	0.0	0.0	4.3	1.9	0.0	0.0	9.1	0.0

C		% of resistant *Staphylococcus aureus*											
Antibiotic category		**Year**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**
		Number of strains (n)	**114**	**163**	**198**	**190**	**226**	**253**	**185**	**178**	**180**	**186**	**139**
Penicillins		PEN	55.3	35.6*	46.5*	41.6	32.3	32.8	44.3*	42.1	39.4	44.1	43.9
		AMP/AMX	55.3	35.6*	46.5*	41.6	32.3	32.8	44.3*	42.1	38.9	44.1	43.2
		OXA	NR	NR	NR	NR	NR	NR	23.8	13.5*	13.9	16.7	15.8
		AMC**	5.3	1.8	12.6*	14.2	12.4	15.8	23.8*	13.5	13.9	16.7	15.8
Cephalosporins	**2nd**	FOX**	NT	NT	NT	NT	NT	NT	30.8	17.4	13.9	18.3	17.3
	3rd	CEF**	0.9	2.5	11.1*	13.2	11.5	16.2	24.3*	13.5*	13.9	16.7	15.8
	4th	CEQ**	1.8	4.3	9.1	12.1	11.5	15.8	24.3*	13.5*	13.9	16.7	15.1
Aminoglycosides		STR**	38.6	31.3	57.6*	65.3	43.4*	27.3*	25.9	15.2*	15.0	19.4	20.9
		KAN	NT	NT	NT	NT	NT	NT	24.3	18.5	19.4	25.8	23.0
		GEN**	10.5	7.4	15.2*	18.4	15.0	21.3	23.2	18.5	18.9	25.3	21.6
		AMK	10.5	7.4	15.7	18.4	15.0	21.3	0.0	0.0	0.0	0.0	0.0
Tetracyclines		TET/OT**	18.4	8.6*	18.7*	18.4	17.3	16.6	28.1*	20.2	22.8	29.6	27.3
Macrolides		ERY**	6.1	5.5	12.6*	10.5	6.2	5.9	7.0	3.4	6.1	4.8	5.8
Rifampicin		RIF**	4.4	4.3	11.1*	7.4	5.8	10.3	7.6	2.8*	2.8	2.7	2.9
Sulphonamides		SUL/SXT	0.9	4.3	11.1	7.9	4.9	4.7	9.2	5.1	3.3	2.2	6.5*
Fluoroquinolones		ENO**	0.0	1.2	2.5	2.6	2.7	4.0	8.6*	4.5	5.6	3.8	1.4
		MAR**	0.0	0.6	0.5	1.1	1.3	2.8	2.2	1.1	3.9	2.7	1.4

D	% of resistant *Pseudomonas aeruginosa*										
Antibiotic category	**Year**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**
	Number of strains (n)	**50**	**93**	**75**	**102**	**90**	**127**	**153**	**94**	**95**	**96**	**59**
Cephalosporin 4th	CEQ**	44.0	52.7	34.7*	41.2	34.4	33.1	43.8	46.8	50.5	21.9*	11.9
Aminoglycosides	GEN	14.0	12.9	36.0*	44.1	20.0*	51.2*	58.8	35.1*	20.0*	3.1*	10.2
	AMK**	10.0	7.5	6.7	14.7	6.7	8.7	8.5	10.6	1.1*	1.0	0.0
Fluoroquinolones	MAR	2.0	2.2	2.7	14.7*	8.9	6.3	24.2*	9.6*	3.2	3.1	1.7

E		% of resistant *Escherichia coli*										
Antibiotic category		**Year**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**
		Number of strains (n)	**350**	**419**	**479**	**521**	**467**	**528**	**577**	**490**	**491**	**493**	**344**
Penicillins		AMP/AMX	40.6	33.9	54.1*	56.6	31.3*	52.5*	62.7*	55.7*	39.7*	40.0	39.5
		AMC	28.9	23.6	48.6*	53.0	25.1*	43.6*	45.4	46.1	32.8*	34.9	31.4
Cephalosporins	**3rd**	CEF	3.4	6.7*	8.6	8.6	8.8	10.8	10.2	9.8	11.2	6.7*	6.1
	4th	CEQ**	1.4	6.0*	8.4	8.3	7.9	10.6	9.9	9.2	11.0	6.9*	5.8
Aminoglycosides		STR**	77.1	81.9	88.3	94.8*	90.4*	63.6*	76.1*	65.1*	59.3	36.1	33.1
		KAN	NT	NT	NT	NT	NT	NT	25.3	24.9	21.4	8.9	9.0
		GEN	5.7	8.1*	9.4	11.5	10.5	15.9*	11.8	11.6	10.4	6.9	6.1
		AMK**	3.4	3.8	2.7	3.8	3.4	0.6*	0.5	1.6	1.0	0.4	0.0
Tetracyclines		TET/OT	21.4	20.8	22.8	26.3	24.0	23.9	24.1	20.4	21.4	24.5	20.6
Sulphonamides		SUL/SXT**	24.6	26.7	27.1	28.4	26.1	29.2	62.7*	25.1*	30.1	28.2	31.4
Quinolones/Fluoroquinolones		NAL	NT	NT	NT	NT	NT	NT	7.1	10.0	8.1	6.7	4.9
		FLU**	7.7	17.2*	6.1	7.7	8.1	7.8	6.1	6.3	6.5	6.1	4.9
		ENO	4.0	5.0	5.0	4.6	6.0	6.3	5.9	5.5	6.1	5.3	3.2
		MAR	3.4	3.8	3.5	3.8	4.3	4.9	4.7	3.5	5.1	3.7	2.9

F		% of resistant *Enterobacter spp*										
Antibiotic category		**Year**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**
		Number of strains (n)	**59**	**113**	**103**	**95**	**80**	**75**	**110**	**75**	**69**	**61**	**38**
Cephalosporins	**3rd**	CEF	6.8	18.6	18.4	20.0	10.0	18.7	28.2	12.0*	8.7	16.4	15.8
	4th	CEQ	3.4	8.0	9.7	7.4	8.8*	8.0	11.8*	8.0	1.4	8.2	13.2

Aminoglycosides	STR**	32.2	39.8	62.1*	64.2	47.5	33.3	50.0	29.3	18.8	26.2	23.7
	KAN**	NT	NT	NT	NT	NT	NT	37.3	16.0*	10.1	23.0	18.4
	GEN	3.4	17.7*	23.3	18.9	20.0	24.0	35.5	16.0*	8.7	18.0	18.4
	AMK	1.7	4.4	3.9	9.5	5.0	9.3	8.2	0.0*	1.4	6.6	5.3
Tetracyclines	TET/OT**	10.2	19.5	43.7*	40.0	37.5	26.7	56.4*	38.7*	43.5*	16.4	21.1
Sulphonamides	SUL/SXT	5.1	23.0*	21.4	18.9	18.8	20.0	43.6*	16.0	15.9	19.7	21.1
Quinolones/Fluoroquinolones	NAL	NT	NT	NT	NT	NT	NT	23.6	20.0	20.3	18.0	21.1
	FLU**	23.7	37.2	33.0	23.2	20.0	21.3	24.5	18.7	18.8	18.0	21.1
	ENO	1.7	19.5*	14.6	13.7	10.0	14.7	20.0	10.7	8.7	14.8	7.9
	MAR	0.0	0.9	2.9	0.0	0.0	2.7	1.8	2.7	0.0	4.9	2.6

G		% of resistant *Klebsiella pneumoniae*										
Antibiotic category	**Year**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**
	Number of strains (n)	**55**	**47**	**63**	**58**	**64**	**61**	**66**	**62**	**62**	**57**	**31**
Penicillins	AMC**	18.2	23.4	14.3	24.1	14.1	8.2	10.6	9.7	9.7	7.0	12.9
Cephalosporins 3rd	CEF	9.1	6.4	3.2	10.3	7.8	1.6	6.1	4.8	1.6	7.0	9.7
Cephalosporins 4th	CEQ	5.5	4.3	1.6	1.7	6.3	1.6	4.5	3.2	1.6	7.0	9.7
Aminoglycosides	STR**	40.0	29.8	61.9*	72.4	56.3	23.0*	21.2	25.8	19.4	26.3	29.0
	KAN	NT	NT	NT	NT	NT	NT	6.1	1.6	0.0	3.5	3.2
	GEN	9.1	10.6	3.2	3.4	4.7	3.3	9.1	3.2	0.0	3.5	6.5
	AMK	0.0	0.0	0.0	0.0	0.0	0.0	1.5	0.0	0.0	0.0	0.0
Tetracyclines	TET/OTC	12.7	10.6	30.2*	41.4	15.6*	16.4	27.3	27.4	24.2	12.3	25.8
Sulphonamides	SUL/SXT**	18.2	12.8	3.2	10.3	9.4	8.2	21.2	11.3	12.9	19.3	32.3
Quinolones/Fluoroquinolones	NAL	NT	NT	NT	NT	NT	NT	13.6	32.3*	12.9*	5.3	19.4*
	FLU	3.6	12.8	1.6*	13.8*	7.8	3.3	9.1	8.1	6.5	3.5	12.9
	ENO**	1.8	2.1	0.0	3.4	1.6	0.0	9.1*	3.2	4.8	3.5	9.7
	MAR	0.0	0.0	0.0	0.0	1.6	0.0	6.1	0.0	1.6	0.0	3.2

$R \leq 10\%$ | $10\% < R \leq 30\%$ | $30\% < R \leq 50\%$ | $R > 50\%$

PEN, penicillin; AMP/AMX, ampicillin or amoxicillin; AMC, amoxicillin-clavulanic acid; CEF, ceftiofur; CEQ, cefquinome; STR, streptomycin; KAN, kanamycin; GEN, gentamicin; TET/OT, tetracycline or oxytetracycline; ERY, erythromycin; RIF, rifampicin; SUL/SXT, sulphonamides or trimethoprim-sulfamethoxazole; ENO, enrofloxacin; MAR, marbofloxacin; FLU, flumequine; OXA, oxacillin, marker of methicillin resistance; FOX, cefoxitin, marker of cephalosporin resistance; NAL, nalidixic acid, marker of fluoroquinolone resistance; NT, not tested; NR, not registered; HC, high concentration.
*χ^2 test, *P*-value <0.05.
**Cochran-Armitage trend test, *P*-value <0.05.
Green corresponds to R≤10%, Yellow to 10<R≤30%, Pink to 30<R≤50% and Red to R>50%.

Susceptibility Testing (EUCAST; http://www.eucast.org). After 18–24 hours of incubation at 37 °C, diameters of growth inhibition around the discs were measured using OSIRIS (Bio-Rad, Marnes-la-Coquette, France) or SIRSCAN (I2A, Montpellier, France) and interpreted as susceptible, intermediate or resistant, according to EUCAST clinical breakpoints. Of note, bacteria that were categorised as intermediate were further considered as resistant. Bacterial isolates were evaluated for their susceptibilities to β-lactams, polymyxins, aminoglycosides, tetracyclines, macrolides, rifampicin, sulphonamides and fluoroquinolones (Table S1). Until November 2011, AST was performed without Gram distinction, whereas from December 2011 two different panels were tested:

one for Gram-positives and another for Gram-negatives (Table S1). The sulphonamides were tested alone just from December 2011 to December 2012 because the combination of trimethoprim and sulfamethoxazole was used together by veterinarians. Amikacin was systematically tested until July 2016, then only if other antimicrobial compounds were inactive on Gram-negative bacteria.

2.4. Statistical analysis

Statistical analysis was performed using the XLStat software. The χ^2 test was undertaken to test for significant changes in antimicrobial resistance among each bacterial species over the period. The temporal trends in prevalence of antimicrobial resistance were investigated for each antimicrobial agent using the Cochran Armitage trend test. For these analyses, P values <0.05 were considered as significant.

3. Results

3.1. Origin and identification of equine clinical isolates

Out of 25 813 isolates, respiratory isolates represented the most important group (n = 9512; 36.8%), followed by genital (n = 7689; 29.8%), cutaneous (n = 4083; 15.8%), and other isolates (n = 4529; 17.6%).

The most frequently encountered bacterial species responsible for horse infections were group C *Streptococci* (27.6%), *Escherichia coli* (*E. coli*, 20.0%), *Staphylococcus aureus* (*S. aureus*, 7.8%), *Pseudomonas aeruginosa* (*P. aeruginosa*, 4.0%), *Enterobacter* spp. (3.4%), *Klebsiella pneumoniae* (*K. pneumoniae*, 2.4%) and *Rhodococcus equi* (*R. equi*, 1.8%) (Fig. 1A). Their distributions according to the source of infection group are presented in Fig. 1B.

3.2. Antimicrobial susceptibility of equine pathogens

Group C *Streptococci* were the most frequently isolated bacteria (27.6%) mainly from genital (41.1%) and respiratory (30.6%) samples (Fig. 1). Among the 7124 clinical isolates, it was observed that penicillins remained active against all streptococcal isolates, since no resistant phenotype was observed during this period (Table 1A). Tetracycline was the only antimicrobial for which the number of resistant bacteria significantly increased between 2006–2016, with 22.0% and 82.0% of resistant strains, respectively (Table 1A). Moreover, as shown in Fig. 2A, most of these tetracycline-resistant strains came from genital origin. Moreover, <23.0% of the group C *Streptococci* were resistant to aminoglycosides, macrolides, rifampicin and sulphonamides. Because *Streptococci* are intrinsically resistant to low levels of aminoglycosides, high concentration (HC) discs of aminoglycosides were used for AST, and <3.0% of group C *Streptococci* were categorised as highly resistant to gentamicin HC. As for tetracycline-resistant isolates, it was observed that strains resistant to aminoglycosides and macrolides were mainly isolated from genital samples: 54.8% (397 of 724) and 57.3% (351 of 612), respectively (Fig. 2A, Table S2).

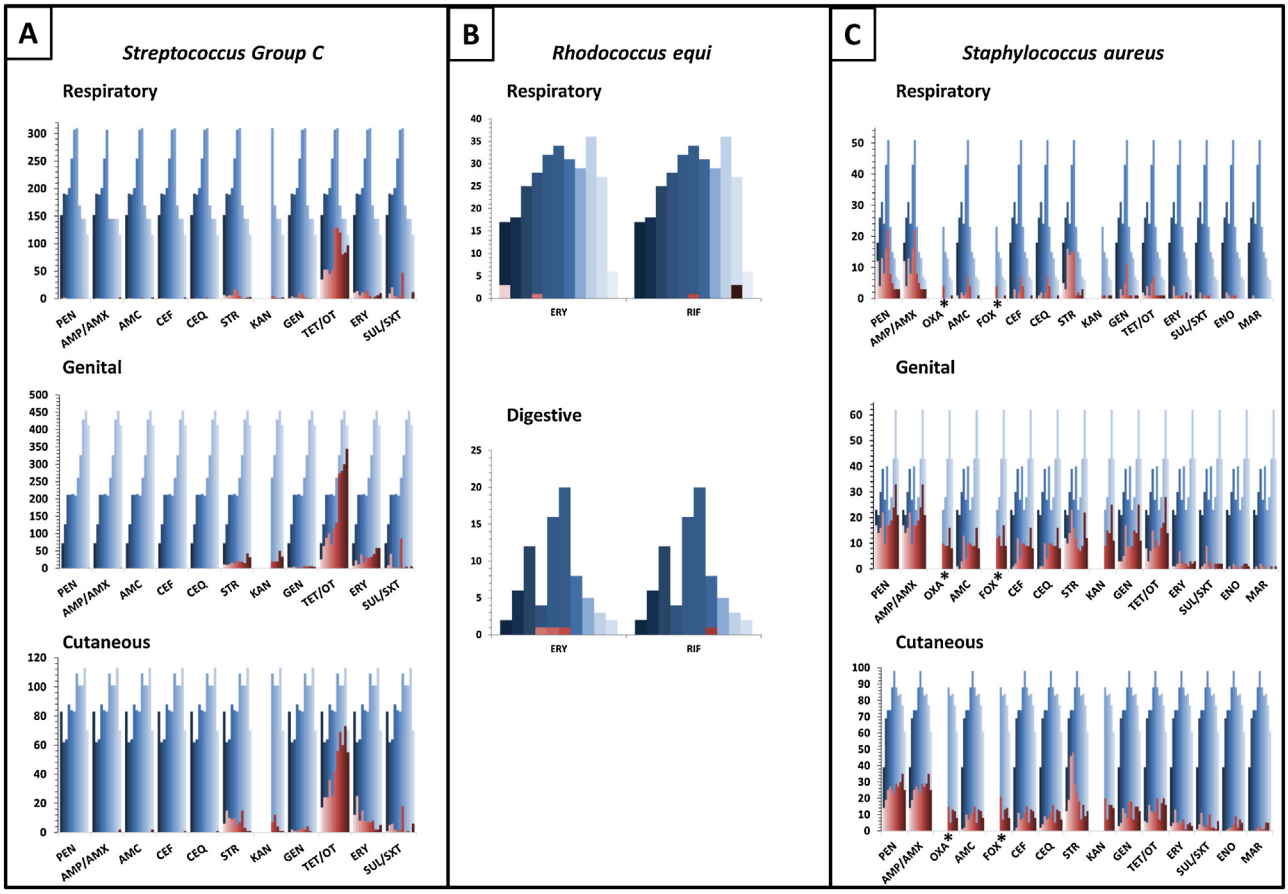

Fig. 2. Total (blue) and resistant (red) relevant Gram-positive (A, B, C) and Gram-negative (D, E, F, G) bacteria isolated per year and sample group.
PEN, penicillin; AMP/AMX, ampicillin or amoxicillin; AMC, amoxicillin-clavulanic acid; CEF, ceftiofur; CEQ, cefquinome; STR, streptomycin; KAN, kanamycin; GEN, gentamicin; TET/OT, tetracycline or oxytetracycline; ERY, erythromycin; RIF, rifampicin; SUL/SXT, sulphonamide or trimethoprim-sulfamethoxazole; ENO, enrofloxacin; MAR, marbofloxacin; FLU, flumequine; OXA*, oxacillin, marker of methicillin resistance; FOX*, cefoxitin, marker of cephalosporin resistance; NAL*, nalidixic acid, marker of fluoroquinolone resistance.

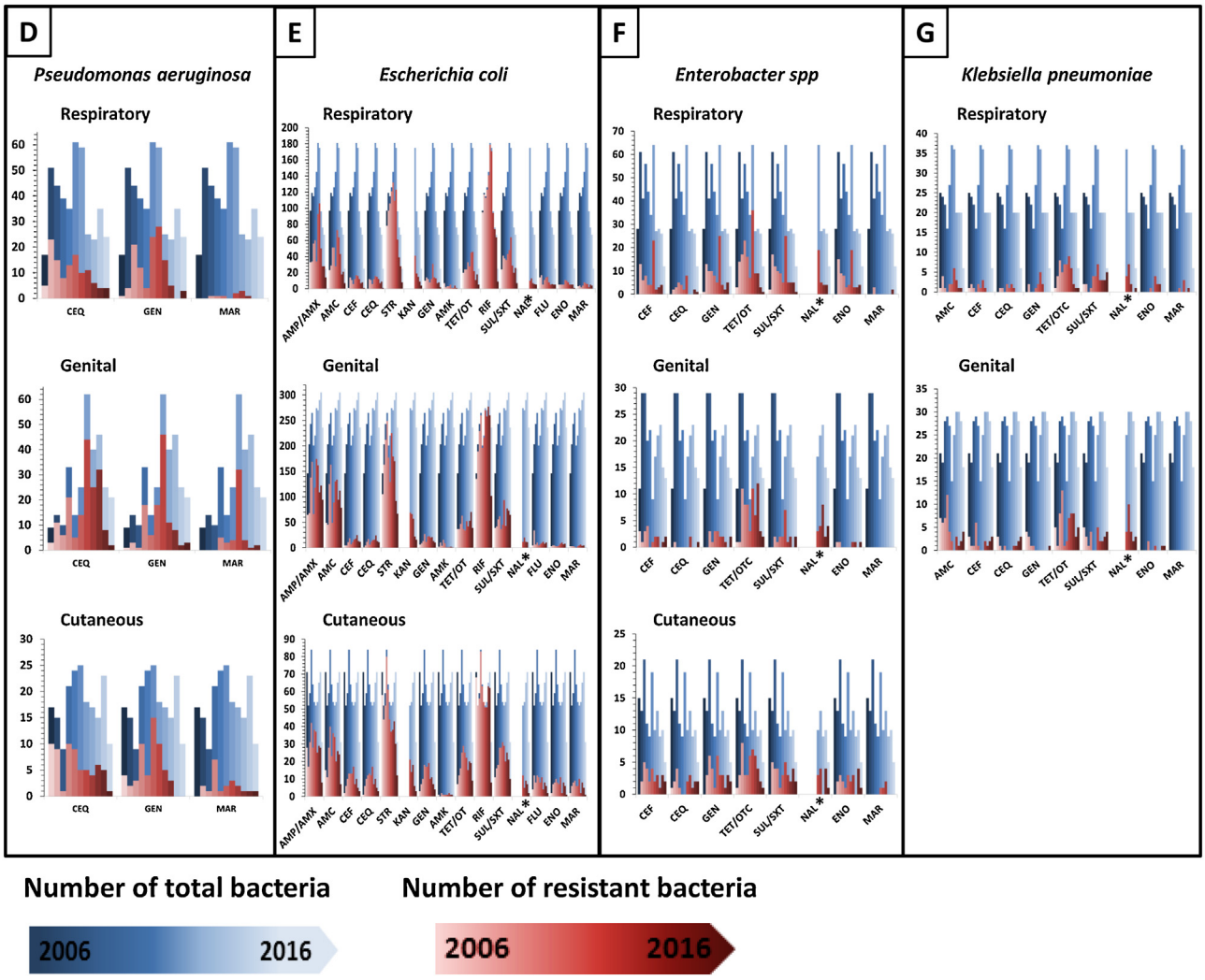

Fig. 2. (Continued)

Rhodococcus equi represented 1.8% of total bacteria and was, as expected, mainly isolated from respiratory (61.3%) samples (Fig. 1). Note that in *R. equi* strains from the group 'other', >47.0% were from digestive origin (Table S2). As the treatment of *R. equi* infection is based on the use of a combination of macrolides and rifampicin, only the resistance of these two antibiotics was studied (Fig. 2B). No increase in resistance of *R. equi* to erythromycin and rifampicin was observed over the 11-year study period (Table 1B).

Staphylococcus aureus represented near 8.0% of total bacteria isolated in infected horses between 2006–2016 (Fig. 1A). As shown in Fig. 1B, most of *S. aureus* were isolated from cutaneous (41.5%), genital (18.8%) and respiratory (12.8%) samples. During this period, 32–55.0% of the samples were resistant to penicillin and ampicillin, whereas 99–77.0% remained susceptible to ampicillin when clavulanic acid was present (Table 1C). The levels of *S. aureus* resistant to oxacillin and cefoxitin (used as markers for methicillin resistance from 2012 and extended to other ß-lactams) were around 17.0% (except in 2012 with 30.0%). Thus, it appears that this evolution stopped in 2013, followed by a stable level of methicillin-resistant *S. aureus* (MRSA) around 17.0% (Table 1C). In addition, except for the high level of streptomycin-resistant strains isolated between 2006 and 2010 (>30.0%), <30.0% of the strains were resistant to the other aminoglycosides, macrolides, rifampicin, sulphonamides and quinolones (Table 1C). Based on the samples' origin, those from the respiratory tract contained less resistant-strains of *S. aureus* (12.8%; 257 of 2012) than the others, with 18.8% (379 of 2012) and 41.5% (835 of 2012) for genital and cutaneous tract, respectively (Fig. 2C, Table S2). *Pseudomonas aeruginosa* represented 4.0% of total bacteria and was mainly isolated from respiratory (39.9%) and genital (28.9%) samples (Fig. 1). As only cefquinome (C4G), gentamicin, amikacin and marbofloxacin are clinically relevant, these four antimicrobial agents were included in this study (Table 1D). As shown in Fig. 2D and Table S2, the majority of resistant strains were obtained from respiratory tract samples (39.9%; 413 of 1034) followed by genital swabs (28.9%; 299 of 1034) and cutaneous samples (18.8%; 194 of 1034).

Escherichia coli was the second most frequently isolated bacteria (20%) mainly from genital (51.4%), respiratory (23.7%) and cutaneous (12.7%) samples (Fig. 1). The majority of resistant *E. coli* strains were isolated from genital samples (51.4%; 2653 of 5159) (Fig. 2E, Table S2). Streptomycin was the antimicrobial for which the highest number of resistant bacteria were observed in the 11 years. However, this number decreased from 76.0% of *E. coli* resistant to streptomycin in 2012 to 33.0% in 2016 (Table 1E). Overall, <12.0% of *E. coli* strains were resistant to ceftiofur (C3G), cefquinome (C4G) and fluoroquinolones; and around 40.0% of *E. coli* strains were resistant to amoxicillin.

Enterobacter spp. represented 3.4% of total bacteria and was mainly isolated from respiratory (48.0%), genital (24.0%) and cutaneous (15.0%) samples (Fig. 1). Among the aminoglycosides,

streptomycin was the antimicrobial for which the largest number of resistant strains was observed. Between 2006 and 2012, the level of streptomycin-resistant *Enterobacter spp*. oscillated between 32.0% and 64.0%, after which time it stayed <30.0% (Table 1F). Tetracyclines were the second antimicrobial for which an important level of resistance was observed, especially in 2008–2010 and 2012–2014. During these periods, the percentages of resistant strains could reach approximately 37.0–56.0% (Table 1F). Moreover, <10.0% of the strains were resistant to amikacin and marbofloxacin. When the distribution of resistant strains according to the sample origins was examined, it was observed that the cutaneous group contained the least number of resistant bacteria (15.4%; 135 of 878) compared with the respiratory (47.9%; 421 of 878) and genital samples (23.8%; 208 of 878) (Fig. 2F, Table S2).

Klebsiella pneumoniae represented 2.4% of all the bacteria and were mainly isolated from genital (43.0%) and respiratory (40.0%) samples (Fig. 1). Among the isolated *K. pneumoniae*, the level of resistant bacteria was never >24.0% for amoxicillin-clavulanic acid and 10.0% for cephalosporins and aminoglycosides, with the exception for streptomycin (Table 1G). As for *Enterobacter*, streptomycin was the only aminoglycoside for which a high level of resistance was observed. In 2009, this level reached 72.0% but remained <30.0% since 2011. Sporadic high levels of resistances (>30.0%) were observed for tetracyclines (2008 and 2009), sulphonamides (2016) and nalidixic acid (2013). Genital and respiratory samples contained most of the resistant strains, 43.1% (270 of 626) and 40.4% (253 of 626), respectively (Fig. 2G, Table S2).

3.3. Multidrug-resistant bacteria

Table 2 shows the overall level and trend of the presence of MDR bacteria (defined as non-susceptible to at least three different classes of antibiotic that are usually efficient) according to bacterial species. Because few antimicrobial compounds have been tested against *P. aeruginosa* and *R. equi*, these species were excluded from the analysis. It is worth noting that for *E. coli* and *Enterobacter* spp., the level of MDR remained similar during the 2006–2012 period (average of resistance was 35.7% and 36.1%, respectively), followed by a decrease from 2013–2016 (average of resistance was 23.3% and 25.3%, respectively). For *K. pneumoniae*, the level of MDR varied from 17–39.7% within the 2006–2016 period, with a relative stability around 21% with the exception of 2009 (39.7%), 2013 (35.5%) and 2016 (38.7%). Moreover, for group C *Streptococci*, the level of MDR decreased from 20.4% in 2006 to 9% in 2011 and then to 7% in the 2013–2015 period, with an increase only observed in 2012 (13.8%) and 2016 (10.7%). Finally, the percentage of MDR for *S. aureus* was 43% in 2006; after a decrease to 24.5% in 2007, this level increased to 41.1% in 2009. After 2009, from 18% to 27% of *S. aureus* strains were MDR.

4. Discussion

This large-scale study included >25 000 clinical isolates from equine origin harvested over 11 years. It revealed an important panorama of the antimicrobial resistance of group C *Streptococci*, *Rhodococcus equi*, *Pseudomonas aeruginosa*, Enterobacteria and *Staphylococcus aureus*. The identified group C *Streptococci* included *Streptococcus dysgalactiae* subsp. *equisimilis* and the two most common streptococcal species from horses: *Streptococcus equi* subsp. *zooepidemicus* (which is part of the upper respiratory tract microbiota and can be virulent when it invades the lower respiratory tract), and *Streptococcus equi* subsp. *equi* (the causative agent of strangles) [8]. These observations are in accordance with those made by Johns and Adams (2015), where no resistance to penicillins and increased resistance to tetracyclines were observed [7]. In contrast with the South African and English studies performed between 1999–2012 and 2007–2013, respectively, no increase in gentamicin resistance was observed over the current 11-year study period [3,7]. In the current study, <3.0% of group C *Streptococci* were categorised as resistant, while >70.0% of streptococcal isolates were resistant in the South African study (n = 286) [3]. The reason for this important discordance needs to be still elucidated.

Rhodococcus equi is a soil organism carried in the gut of herbivores. This facultative intracellular pathogen is the most common cause of severe pneumonia in foals [9]. In the past two decades, *R. equi* has emerged as an opportunistic human pathogen that affects immunocompromised patients [10]. Infections caused by this species are clinically well characterised in horses and the treatment is based on the use of a combination of macrolides and rifampicin. Interestingly, as opposed to the literature [11–13], no increase in resistance of *R. equi* to these two antimicrobial agents was observed in the current study.

Staphylococcus aureus is part of the normal cutaneous flora in human and animals. However, *S. aureus* is a major cause of nosocomial and community-acquired infections in France and worldwide [14]. This opportunistic pathogen leads to several pathologies, with various degree of severity [14]. The current results were lower than those reported in the literature, where >60.0% of staphylococci from equine samples were resistant to penicillin [3,6]. In a recent study, 1396 *S. aureus* strains isolated from horses between 2007–2013 were genetically analysed. In this population, 6% of the strains were resistant to methicillin [15]. Over the analysed period (2007–2013) an increased MRSA rate was observed, with 1.0% in 2007 to 11.0% in 2011 [15]. Since 2013, the current results revealed that the level of such resistance was relatively stable around 17%.

Pseudomonas aeruginosa is a major opportunistic pathogen that causes many infections in immunosuppressed humans and people suffering from cystic fibrosis [16]. In horses, it is often associated to endometritis in mares and represents a serious problem due to its

Table 2
Percentage of bacteria resistant to three or more antimicrobial classes.

	Streptococcus (group C)	*Staphylococcus aureus*	*Escherichia coli*	*Enterobacter spp.*	*Klebsiella pneumoniae*
2006	20.4	43.0	34.0	20.3	18.2
2007	14.0	24.5	39.6	31.9	17.0
2008	12.3	38.4	34.2	45.6	23.8
2009	11.6	41.1	38.8	41.1	39.7
2010	6.3	27.0	33.4	36.3	21.9
2011	9.0	27.3	34.1	26.7	21.3
2012	13.8	27.6	31.4	50.9	22.7
2013	6.8	18.0	24.3	24.0	35.5
2014	6.4	20.0	23.6	24.6	22.6
2015	7.5	25.3	22.5	26.2	21.1
2016	10.7	24.5	22.7	26.3	38.7

ability to form biofilm as well as its multi-resistance to antimicrobials [17–19]. As described in the literature, it was observed over the 11 years that few antimicrobial agents remained active against this bacterium as cefquinome (C4G), gentamicin, amikacin and marbofloxacin [3,6].

Escherichia coli is naturally present in the digestive tract of humans and animals and may be responsible for gastrointestinal disorders, urinary tract infections and septicaemia (in rare cases) [20]. The current study observed an important decrease in resistance to streptomycin (from 76% in 2012 to 33% in 2016). Moreover, the level of resistance to amoxicillin (around 40%) was similar to that observed for human clinical samples [21,22]. Less than 10% of *E. coli* strains were resistant to cephalosporins and/or fluoroquinolones.

The use of these drugs is regulated, since they are designated as 'critically important' by the WHO and French government, in order to limit their consumption. Resistance to third-generation and fourth-generation cephalosporins may be related to the production of cephalosporinase, extended spectrum β-lactamase (ESBL) or carbapenemase, and became an increasing therapeutic problem in equine infection by Enterobacteria such as *E. coli* in the same way as for humans [21,22]. In this context, it could be interesting to determine the incidence of enzyme-producing *E. coli* in cephalosporin-resistant strains.

In a large, lower proportion than *E. coli,* two other Enterobacteria were also analysed: *Enterobacter spp*. (3.4%) and *K. pneumoniae* (2.4%). *Enterobacter* species, especially *E. aerogenes* and *E. cloacae,* have been associated with nosocomial infections and can cause numerous pathologies such as pneumonia, septicaemia, and urinary tract and wound infections [23]. Against these isolates, amikacin and marbofloxacin remained the two anti-microbial compounds for which bacteria have the least resistance (<10%). *Klebsiella pneumoniae* is part of the normal urogenital and intestinal flora of humans and animals [24]. In horses, this bacterium can be responsible of metritis, infertility and pneumonia [25].

In the literature, data on susceptibility of *K. pneumoniae* to commonly used antimicrobials in equine medicine are contradictory. The current data agree with the German survey, where isolates from mare uterine infections were very sensitive to commonly used antimicrobials [26], but are in contrast with two other studies that found an important prevalence of multidrug-resistant *Klebsiella* [3,27].

By pooling all data, an overview of MDR was shown. For Enterobacteria, according to the strain and the period, the rate of MDR was from 17–39%. On the other hand, the percentages of MDR strains of group C *Streptococci* decreased to around 7% (in 2013–2015). This level is relatively low but should be maintained with rational use of antimicrobial drugs. Interestingly, 43% of *S. aureus* were MDR in 2006 versus 18–25% after 2012. Except erythromycin, rifampicin and amoxicillin-clavulanate, French equine practitioners usually use all other antimicrobial compounds without any previous microbiological characterisation, increasing the risk of non-adapted treatment.

It could be hypothesised that the reduction in MDR strains of *Staphylococcus* over the study period may be linked to the French national ECOANTIBIO Plan (2012–2016), which aimed to educate practitioners to use antimicrobials with care, and lead to an important decrease (37%) in the exposure of animals to antibiotics. In 2013, Hughes et al. showed that 0.8% (2 of 281) of equine veterinarians in the UK had antimicrobial use guidelines and that 56% of licensed antimicrobial prescriptions were over the recommended dose rate [28]. It is reasonable to believe that empirical practices may largely contribute to the high prevalence of MDR, and the current results argue for the crucial role of information and warnings against the problem of resistant pathogens. It is believed that this work is the first large-scale retrospective study conducted in France over an 11-year period with >25 000 samples analysed for their antimicrobial susceptibilities.

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Competing interest

All other authors declare no competing interest.

Ethical approval

Not required.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2019.03.006.

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