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Original article

From genotype to antibiotic susceptibility phenotype in the order Enterobacterales: a clinical perspective

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ABSTRACT

Objectives: Predicting the antibiotic susceptibility phenotype from genomic data is challenging, especially for some specific antibiotics in the order Enterobacterales. Here we aimed to assess the performance of whole genomic sequencing (WGS) for predicting the antibiotic susceptibility in various Enterobacterales species using the detection of antibiotic resistance genes (ARGs), specific mutations and a knowledge-based decision algorithm.
Methods: We sequenced (Illumina MiSeq, 2×250 bp) 187 clinical isolates from species possessing ($n = 98$) or not ($n = 89$) an intrinsic AmpC-type cephalosporinase. Phenotypic antibiotic susceptibility was performed by the disc diffusion method. Reads were assembled by A5-miseq and ARGs were identified from the ResFinder database using Diamond. Mutations on GyrA and ParC topoisomerases were studied. Piperacillin, piperacillin-tazobactam, ceftazidime, cefepime, meropenem, amikacin, gentamicin and ciprofloxacin were considered for prediction.
Results: A total of 1496 isolate/antibiotic combinations (187 isolates × 8 antibiotics) were considered. In 230 cases (15.4%), no attempt of prediction was made because it could not be supported by current knowledge. Among the 1266 attempts, 1220 (96.4%) were correct (963 for predicting susceptibility and 257 for predicting resistance), 24 (1.9%) were major errors (MEs) and 22 (1.7%) were very major errors (VMEs). Concordance were similar between non-AmpC and AmpC-producing Enterobacterales (754/784 (96.2%) vs 466/482 (96.7%), chi-square test p 0.15), but more VMEs were observed in non-AmpC producing strains than in those producing an AmpC (19/784 (2.4%) vs 3/466 (0.6%), chi-square test p 0.02). The majority of VMEs were putatively due to the overexpression of chromosomal genes.
Conclusions: In conclusion, the inference of antibiotic susceptibility from genomic data showed good performances for non-AmpC and AmpC-producing Enterobacterales species. However, more knowledge about the mechanisms underlying the derepression of AmpC are needed. **E. Ruppé, Clin Microbiol Infect 2019;▪:1**

Introduction

Whole genome sequencing (WGS) of bacterial strains has now become the preferred method for the identification of antibiotic resistance determinants, including antibiotic resistance genes (ARGs) and intrinsic genes in which mutational events can lead to antibiotic resistance. Yet, the *in silico* translation from genotype to phenotype may be challenging because it relies on the quality and exhaustiveness of the available knowledge about the genomic determinants of resistance. Several ARG databases have been released over the last decade [1], the most popular ones being ResFinder [2] and CARD [3]. To date though, there is no consensus on which database should be used for inferring antibiotic susceptibility

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phenotype from WGS data. Moreover, the resistance pattern conferred by the ARGs needs to be known, which is not the case for some variants that have not been experimentally tested (i.e. mutations of unknown phenotypic significance). Of note, no database includes phenotypic data associated with the ARG sequence and the resistance phenotype conferred by the presence of an ARG must be inferred from literature. Even more incomplete are the data relative to the mutational events associated with antibiotic resistance such as an increased expression of an intrinsic resistance gene (e.g. bla_{AmpC} in Enterobacterales) and/or a decreased expression of a gene (e.g. *oprD* in *Pseudomonas aeruginosa*).

Nonetheless, the link between the content of antibiotic resistance determinants (referred to as the 'genotype') and the antibiotic resistance profile (the 'phenotype') has been assessed for *Staphylococcus aureus* [4–7], *Escherichia coli* [8–11], *Shigella sonnei* [12], *Klebsiella pneumoniae* [9,13], *P. aeruginosa* [14–16], *Mycobacterium tuberculosis* [17] and *Neisseria gonorrhoeae* [18]. As for *E. coli*/*S. sonnei* and *K. pneumoniae*, the performances of WGS to predict the susceptibility were excellent for β-lactams, fluoroquinolones and aminoglycosides [19].

The use of genomics data to infer antibiotic susceptibility is crucial to the development of clinical metagenomics (CMg), which refers to the metagenomic sequencing of nucleic acids from clinical samples in order to obtain information of clinical relevance [20]. CMg is an emerging field that could transform the way infectious diseases are currently diagnosed. The application of CMg to various clinical samples has shown promising results in the identification of bacterial pathogens, including the ones that are difficult to culture. The identification of ARGs and the inference of antibiotic susceptibility should be integrated in CMg to provide a more complete bacteriological analysis. In the context of severe infections such as sepsis, an early identification of infection-causing bacteria and the inference of its antibiotic susceptibility pattern should improve the patient's care in optimizing the probabilistic regimen earlier [21]. Here we aimed at assessing the performances of WGS to infer the susceptibility of various species of Enterobacterales to the antibiotics commonly used in probabilistic therapy of sepsis.

Material and methods

Selection of the strains

A total of 187 Enterobacterales strains from the bacteriology laboratory of the Geneva University Hospitals (HUG) have been analysed for this project (Fig. S1). All had been isolated from the culture of clinical samples. At least ten strains per were selected among non-AmpC-producing Enterobacterales (*Citrobacter koseri*, *E. coli*, *Klebsiella oxytoca*, *K. pneumoniae, Proteus vulgaris*, *Proteus mirabilis*) and among AmpC-producing Enterobacterales (*Citrobacter freundii*, *Klebsiella aerogenes*, *Enterobacter cloacae*, *Hafnia alvei*, *Morganella morganii*, *Providencia stuartii*, *Serratia marcescens*). Only one (most recently isolated) strain per patient was selected. For *E. coli* and *K. pneumoniae*, multidrug-resistant strains (producing extended-spectrum β-lactamases (ESBL) and/or carbapenemases) were also specifically chosen. Strain identification was performed using whole-cell matrix-assisted desorption ionization-time of flight mass spectrometry analysis (MALDI-TOF MS; Maldi Biotyper compass, Bruker Daltonics, Bremen, Germany) according to the manufacturer's instructions.

The antibiotic susceptibility testing was performed using the disc diffusion test method and the results interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST v6.0) methods. Intermediate-susceptibility results were considered resistant in this study. The minimal inhibitory concentrations (MICs) were determined *a posteriori* in case of discrepancies using gradient diffusion or broth microdilution. The antibiotics considered for this study were penicillins (piperacillin), penicillins+β-lactamases inhibitors (piperacillin-tazobactam), cephalosporins (ceftazidime and cefepime), carbapenems (meropenem), aminoglycosides (gentamicin and amikacin) and fluoroquinolones (ciprofloxacin). No ethics clearance was issued for this work according to the local laws.

DNA extraction and genome sequencing

Genomic DNA of each isolate was extracted from colonies grown overnight at 37°C on blood agar plates using the MagCore Genomic DNA Tissue Kit (RBC Bioscience, New Taipei City, Taiwan), as described previously [22]. Purified DNA was sent to Fasteris (Plan-les-Ouates, Switzerland) for sequencing. The library was prepared using the Nextera XT DNA Sample Preparation Kit according to the Illumina (San Diego, CA) instructions, and was sequenced on an Illumina MiSeq with 2 × 250 cycles. Six sequencing runs were performed, each including 21–50 multiplexed samples.

Bioinformatic analyses

The bioinformatic analyses are detailed in the supplementary material and in Figs. S2, S3 and S4. Briefly, raw reads were trimmed and quality-filtered using Trimmomatic [23] before being assembled using the A5-miseq assembler [24] (version 20160825:243). ARGs were sought using Diamond [25] and the ResFinder database, accessed October 2017 [2]. The threshold for ARG positive detection was arbitrarily set at an amino acid identity of ≥80% and a query coverage ≥80%.

Genotype to phenotype inference

To determine links between genotype and phenotype, we applied specific rules related to the species, the presence of ARGs and the antibiotic (Table S1). We assumed that all the ARGs found in the strains were expressed except for some chromosomal ARGs (Table S1). In AmpC-producing species, the presence of a premature stop codon, an insertion or deletion event in *ampD* and/or *ampR* was deemed to be associated to a high level of *ampC* expression. When no mutation leading to an amino acid change in AmpD or AmpR was observed, the isolate was considered to be producing AmpC at a low level. Otherwise (mutations in AmpD and/or AmpR), no prediction for the susceptibility to piperacillin, piperacillin-tazobactam and ceftazidime was attempted, unless another β-lactamase known to confer resistance to those antibiotics was identified. A very major error (VME) was defined as inferring susceptibility from genomic data while the strain was actually resistant by phenotypic tests. A major error (ME) was defined as inferring resistance from genomic data while the strain was actually susceptible by phenotypic tests. In case of VMEs for β-lactam susceptibility profile inference, other β-lactamases were searched in the ResFinderFG database (https://cge.cbs.dtu.dk/services/ResFinderFG/).

Results

Genotype to phenotype inference

The analysis of the genomic sequence of 187 strains revealed 517 ARGs conferring resistance to β-lactams, aminoglycosides or fluoroquinolones, within 82 loci. Overall, 1496 isolate−-antibiotic combinations (187 strains × 8 antibiotics) were considered (Table 1). In 230 cases (15.4%), only in AmpC-producing species, no attempt of antibiotic resistance prediction was made. Among 1266

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Table 1
Detailed results of predictions (in percentages) by species

Inducible AmpC	Species	Number of strains	PIP				TZP				CAZ				FEP				MEM				GEN				AMK				CIP			
			C(R)	C(S)	ME	VME	NP	C(R)	C(S)	ME	VME	NP	C(R)	C(S)	ME	VME	NP	C(R)	C(S)	ME	VME	NP	C(R)	C(S)	ME	VME	NP	C(R)	C(S)	ME	VME	NP	C(R)	C(S)	ME	VME	NP
No	*Citrobacter koseri*	24	100	0	0	0	0	0	100	0	0	0	0	100	0	0	0	0	100	0	0	0	0	100	0	0	0	0	0	100	0	0	0	0	0	0
	Escherichia coli	17	100	0	0	0	0	53	35	0	12	0	71	29	0	0	0	47	47	0	6	0	6	94	0	0	0	0	29	71	0	0	0	76	24	0	0	0
	Klebsiella oxytoca	11	100	0	0	0	0	0	82	0	18	0	9	91	0	0	0	9	91	0	0	0	0	100	0	0	0	0	9	91	0	0	9	9	91	0	0	0
	Klebsiella pneumoniae	15	100	0	0	0	0	0	40	13	0	47	0	73	13	0	13	0	73	27	0	0	0	20	80	0	0	0	0	60	40	0	0	80	20	0	0	0
	Proteus vulgaris	17	0	100	0	0	0	0	0	100	0	0	0	0	100	0	0	0	0	100	0	0	0	0	0	100	0	0	0	0	100	0	0	0	88	12	0	0
	Proteus mirabilis	14	36	50	0	14	0	0	0	93	0	7	0	7	86	0	7	0	7	93	0	0	0	0	0	100	0	0	0	0	0	100	0	0	43	50	7	0	0
Yes	*Citrobacter freundii*	14	29	0	0	0	71	21	0	0	0	0	21	0	0	0	79	14	86	0	0	0	79	0	93	0	0	0	0	7	64	29	0	0	7	79	14	0	0
	Klebsiella aerogenes	11	9	9	9	0	73	9	9	9	0	0	9	9	9	0	73	0	91	0	9	0	0	0	91	0	0	0	0	0	100	0	0	0	91	0	0	0	0
	Enterobacter cloacae	13	8	0	0	0	92	8	0	0	0	0	8	0	0	0	92	0	92	0	8	0	0	0	100	0	0	0	0	0	100	0	0	0	92	8	0	0	0
	Hafnia alvei	14	0	14	0	0	86	0	14	0	0	0	14	0	0	0	86	0	100	0	0	0	0	0	100	0	0	0	0	0	100	0	0	0	100	0	0	0	0
	Morganella morganii	14	21	0	0	0	79	0	0	14	0	86	0	0	0	0	100	7	0	0	0	93	0	100	0	0	0	0	0	86	14	0	0	0	79	0	0	0	0
	Providencia stuartii	12	17	0	0	0	83	0	0	0	0	100	17	0	0	0	83	17	83	0	0	0	0	100	0	0	0	0	0	100	0	0	0	0	100	0	0	0	0
	Serratia marcescens	11	0	0	0	0	100	0	0	0	0	100	0	0	0	0	100	0	100	0	0	0	0	100	0	0	0	0	0	100	0	0	0	0	100	0	0	0	0

PIP, piperacillin; TZP, piperacillin-tazobactam; FEP, cefepime; MEM, meropenem; GEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin. C(R), correct prediction of resistance. C(S), correct prediction of susceptibility. ME, major error. VME, very major error. NP, not predicted.

attempts, 1220 (96.4%) were correct (963 for predicting susceptibility and 257 for predicting resistance), 24 (1.9%) were MEs and 22 (1.7%) were VMEs (Fig. 1, Tables 2 and 3). The correctness of predictions was similar between AmpC-producing and non-producing Enterobacterales (754/784 (96.2%) vs 466/482 (96.7%), chi-square test p 0.15), but more VMEs were observed in non-AmpC producing species than in those producing an AmpC (19/784 (2.4%) vs 3/466 (0.6%), chi-square test p 0.02). Detailed results of predictions by species are available in Table 1 and by strains in the Table S2.

Penicillins and combination penicillins with β-lactamase inhibitor

In total, 197 β-lactamases were identified from 47 loci (Fig. S5). The most common were CTX-M-15 and TEM-1. In non-AmpC producing species, the predictions were 98.0% correct for piperacillin and 87.8% for piperacillin-tazobactam (Fig. 1). Two VMEs were found for piperacillin. The strain *P. mirabilis* 75 was resistant to penicillins, penicillins+β-lactamases inhibitors and to extended-spectrum cephalosporins by phenotypic tests; however, no β-lactamase or mutation on penicillin-binding proteins (PBP) were identified by the genome analysis (data not shown). The other VME, found in *P. mirabilis* 122, was due to the production of HMS-1 which is an unpublished β-lactamase ($bla_{\text{HMS-1}}$ being absent from ResFinder while listed in ResFinderFG). For piperacillin-tazobactam, 12 VMEs were found, among which ten were confirmed after MIC determination by broth microdilution. In AmpC-producing species, a prediction was only made for 16.9% (piperacillin) and 12.4% (piperacillin-tazobactam) of strains, and they were correct in 93.3% (14/15) and 72.3% (8/11), respectively.

Extended spectrum cephalosporins (ceftazidime, cefepime) and carbapenems (meropenem)

Among the 197 β-lactamases identified by WGS (Fig. S5), 28 were of extended-spectrum (26 CTX-M, 1 TEM-130 and 1 TEM-138). In non-AmpC strains, the inference of ceftazidime susceptibility was correct in 96.9% (95/98) strains (Fig. 1), leaving three VMEs. In AmpC-producing species, most strains had mutations in AmpD and/or AmpR and the inference of ceftazidime susceptibility could be attempted in only 11/89 strains (12.4%). The prediction was correct in 10/11 (90.9%) strains, with one ME.

A correct inference of cefepime susceptibility was achieved in 97.9% (183/187) of strains (Fig. 1), with four VMEs. In particular, a VME was observed in *E. coli* 53 harbouring a combination of a CMY-42 and a OXA-181, both not being reported to alter cefepime susceptibility. As for meropenem, only one VME was found and a correct inference was found in 99.5% (186/187) strains (Fig. 1).

Aminoglycosides

Resistance to aminoglycosides is due to the acquisition of aminoglycosides modifying enzymes (AMEs) (phosphotransferases (APH), nucleotidyltransferases (ANT) and acetyltransferases (AAC)) and 16S rRNA methyltransferases (e.g. Arm, Rmt). We identified 177 AMEs and one 16S rRNA methyltransferase, from 24 loci (Fig. S6). For gentamicin, all predictions were correct (Fig. 1). For amikacin, 13 MEs (out of which 11 being confirmed after MIC determination) were observed (Fig. 1), with the corresponding strains all carrying an *aac(6′)-Ib-cr* and/or an *aac(6′)-If* but were reported as susceptible to amikacin.

Ciprofloxacin

Fluoroquinolone resistance occurs through mutations in a specific region of the topoisomerases (the quinolone resistance

determining region, QRDR) and/or the acquisition of plasmid-mediated quinolone resistance (PMQR) elements such as Qnr. The distribution of GyrA and ParC topoisomerases mutations in the QRDR is depicted in the Fig. S7. A total of 36 strains had non-synonymous mutations in the QRDR of *gyrA* and/or *parC* genes. Besides, a total of 161 PMQR genes (*qnr* and *aac(6′)-Ib-cr*) and chromosomal efflux pump (OqxA-B) were identified from 11 loci (Fig. S8). A correct inference of susceptibility to ciprofloxacin was obtained in 96.8% (181/187) strain.

Discussion

The main result of this study is the overall high level of correct predictions of antibiotic susceptibility from WGS data when predictions were attempted, with a 96.4% concordance between phenotypic testing and genotypic results. The results obtained in non-AmpC strains were similar to those already published for *E. coli* [8–11] and *K. pneumoniae* [9,13]. Interestingly, similar results were obtained with AmpC-producing species when the inference was

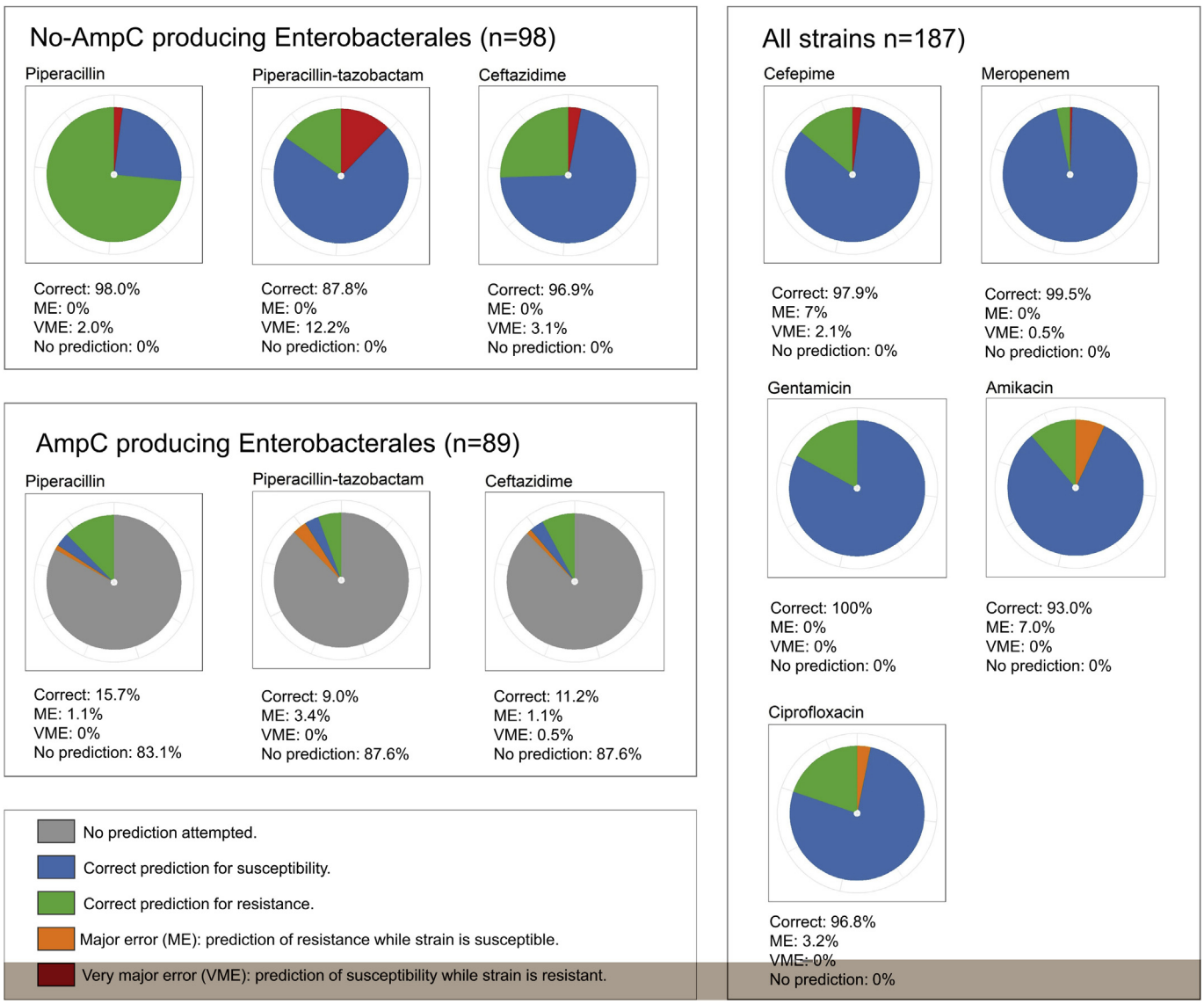

Fig. 1. Performance of genotype to phenotype inference for the 187 strains of the study. ME, major error; VME, very major error. [a]The chi-square test was used with a significance threshold of 0.05.

Table 2
Results of the predictions

Prediction	Non-AmpC strains (n = 784 combinations)	AmpC-strains (n = 712 combinations)	All (n = 1496 combinations)	Difference non-AmpC/AmpC[a]
Attempted	784 (100%[a])	482 (67.7%[a])	1,266 (84.6%[a])	$p = 4.8 \times 10^{-67}$
Correct	754 (96.2%[b])	466 (96.7%[b])	1,220 (96.4%[b])	$p = 0.15$
Major error	11 (1.4%[b])	13 (2.7%[b])	24 (1.9%[b])	$p = 0.1$
Very major error	19 (2.4%[b])	3 (0.6%[b])	22 (1.7%[b])	$p = 0.02$

[a] All combinations were considered.
[b] Only attempts were considered.

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Table 3
Details of the errors observed in this study

Antibiotic	Type of error	Strain	Antibiotic resistance gene(s)	Possible explanation
Piperacillin	Very major error	*Proteus mirabilis* 122	None identified	Production of $bla_{\text{HMS-1}}$ (not included in ResFinder)
Piperacillin, piperacillin-tazobactam, ceftazidime	Major error	*Klebsiella aerogenes* 102	$bla_{\text{CMY-70}}$	Deletion in *ampD* did not lead to the derepression of bla_{AmpC}
Piperacillin, piperacillin-tazobactam, ceftazidime, cefepime	Very major error	*P. mirabilis* 75	None identified	None.
Piperacillin-tazobactam	Very major error	*Escherichia coli* 91	$bla_{\text{TEM-1}}$	High-level production of $bla_{\text{TEM-1}}$
Piperacillin-tazobactam	Very major error	*E. coli* 93	$bla_{\text{TEM-1}}$	High-level production of $bla_{\text{TEM-1}}$
Piperacillin-tazobactam	Very major error	*Klebsiella oxytoca* 163	$bla_{\text{OXY-2-8}}$	High-level production of bla_{OXY}
Piperacillin-tazobactam	Very major error	*K. oxytoca* 251	$bla_{\text{OXY-5-2}}$	High-level production of bla_{OXY}
Piperacillin-tazobactam	Very major error	*Klebsiella pneumoniae* 29	$bla_{\text{CTX-M-15}}$, $bla_{\text{SHV-11}}$	High-level production of bla_{SHV}
Piperacillin-tazobactam	Very major error	*K. pneumoniae* 42	$bla_{\text{CTX-M-15}}$, $bla_{\text{SHV-28}}$, $bla_{\text{TEM-1}}$	High-level production of $bla_{\text{TEM-1}}$ and/or bla_{SHV}
Piperacillin-tazobactam	Very major error	*K. pneumoniae* 55	$bla_{\text{CTX-M-15}}$, $bla_{\text{SHV-28}}$, $bla_{\text{TEM-1}}$	High-level production of $bla_{\text{TEM-1}}$ and/or bla_{SHV}
Piperacillin-tazobactam, ceftazidime	Very major error	*K. pneumoniae* 57	$bla_{\text{SHV-1}}$	High-level production of bla_{SHV}
Piperacillin-tazobactam	Very major error	*K. pneumoniae* 58	$bla_{\text{CTX-M-15}}$, $bla_{\text{SHV-28-like}}$, $bla_{\text{TEM-1}}$	High-level production of $bla_{\text{TEM-1}}$ and/or bla_{SHV}
Piperacillin-tazobactam, ceftazidime	Very major error	*K. pneumoniae* 60	$bla_{\text{SHV-1}}$	High-level production of bla_{SHV}
Piperacillin-tazobactam	Very major error	*K. pneumoniae* 87	$bla_{\text{CTX-M-15}}$, $bla_{\text{SHV-76}}$, $bla_{\text{TEM-1}}$	High-level production of $bla_{\text{TEM-1}}$ and/or bla_{SHV}
Piperacillin-tazobactam	Major error	*Morganella morganii* 13	$bla_{\text{DHA-5}}$, $bla_{\text{OXA-1}}$, $bla_{\text{TEM-1}}$	Low level expression of $bla_{\text{OXA-1}}$
Piperacillin-tazobactam	Major error	*M. morganii* 141	$bla_{\text{CTX-M-15}}$, $bla_{\text{DHA-1}}$, $bla_{\text{OXA-1}}$	Low level expression of $bla_{\text{OXA-1}}$
Cefepime	Very major error	*Enterobacter cloacae* 5	$bla_{\text{ACT-6}}$	Derepression of bla_{AmpC} and loss of permeability.
Cefepime	Very major error	*E. coli* 53	$bla_{\text{CMY-42}}$, $bla_{\text{OXA-181}}$	Combination of the expression of $bla_{\text{CMY-42}}$ and $bla_{\text{OXA-181}}$
Cefepime, meropenem	Very major error	*Klebsiella aerogenes* 240	None identified	Derepression of bla_{AmpC} and loss of permeability.
Amikacin	Major error	*M. morganii* 141	*aac(3)-IIa, aac(6′)Ib-cr*	Resistance not detected by the disc diffusion method.
Amikacin	Major error	*Citrobacter freundii* 116	*aac(6′)-If*	Resistance not detected by the disc diffusion method.
Amikacin	Major error	*C. freundii* 165	*aac(6′)-If*	Resistance not detected by the disc diffusion method.
Amikacin	Major error	*C. freundii* 17	*aac(6′)-Ib-cr, aac(6′)-If, aadA1, aph(3′)-Ia*	Resistance not detected by the disc diffusion method.
Amikacin	Major error	*C. freundii* 184	*aac(6′)-If*	Resistance not detected by the disc diffusion method.
Amikacin	Major error	*E. coli* 111	*aac(6′)Ib-cr, aadA5, strA, strB*	Resistance not detected by the disc diffusion method.
Amikacin	Major error	*E. coli* 86	*aac(3)-IIa, aac(6′)-Ib-cr, aadA5, strA, strB*	Resistance not detected by the disc diffusion method.
Amikacin	Major error	*Klebsiella oxytoca* 255	*aac(3)-IIa, aac(6′)-Ib-cr, aadA1, aadA1, strA, strB*	Resistance not detected by the disc diffusion method.
Amikacin	Major error	*K. pneumoniae* 52	*aac(6′)-Ib-cr, aadA5*	Resistance not detected by the disc diffusion method.
Amikacin	Major error	*K. pneumoniae* 55	*aac(3)-IIa, aac(6′)Ib-cr, aadA16, strA, strB*	Resistance not detected by the disc diffusion method.
Amikacin	Major error	*K. pneumoniae* 85	*aac(3)-IIa, aac(6′)-Ib-cr, strA, strB*	Resistance not detected by the disc diffusion method.
Amikacin	Major error	*K. pneumoniae* 58	*aac(3)-IIa, aac(6′)-Ib-cr, aadA16, strA, strB*	Resistance not detected by the disc diffusion method.
Amikacin	Major error	*K. pneumoniae* 87	*aac(3)-IIa, aac(6′)-Ib-cr, strA, strB*	Resistance not detected by the disc diffusion method.
Ciprofloxacin	Major error	*C. freundii* 128	*qnrB72*, *qnrS1*, GyrA_WT, ParC_WT	*qnrS1* expressed at low level.
Ciprofloxacin	Major error	*C. freundii* 190	*qnrB38*, GyrA_T83I, ParC_WT	Mutation in GyrA did not result in detectable resistance.
Ciprofloxacin	Major error	*E. cloacae* 7	*oqxA*, *oqxB*, GyrA_S83T, GyrA_I112V, ParC_WT	Both mutations on GyrA did not result in detectable resistance.
Ciprofloxacin	Major error	*Proteus mirabilis 122*	*qnrD*, GyrA_WT, ParC_WT	*qnrD* expressed at low level.
Ciprofloxacin	Major error	*Proteus vulgaris 155*	*qnrD*, GyrA_WT, ParC_WT	*qnrD* expressed at low level.
Ciprofloxacin	Major error	*P. vulgaris* 194	None, GyrA_WT, ParC_S84G	Mutation in ParC did not result in detectable resistance.

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attempted. However, the prediction of the overexpression of AmpC from genomic data will require more data in order to be effective. Indeed, we found that only a minority of AmpC-producing strains had *ampD* and *ampR* genes highly similar to those of the corresponding reference NCBI genome, which highlights the need for sequencing more strains from AmpC-producing species in order to cover their genetic diversity.

For some antibiotics, WGS could even be more accurate in detecting resistance than conventional testing. The EUCAST guidelines recommend to interpret as non-susceptible to amikacin a strain which is resistant to tobramycin but apparently susceptible to gentamicin and amikacin, i.e. suggesting the production of an AAC(6′)-I enzyme [27]. Nonetheless, the application of this recommendation is compromised by the co-production of an AAC(3)-II which confers resistance to gentamicin and thereby AAC(6′)-I − producing strains may falsely appear to be susceptible to amikacin. For quinolones, we found an acquired *qnr* gene in two strains that were nonetheless susceptible to ciprofloxacin. Such detection could be of interest for clinicians as low-level resistance to quinolones could be associated to a decreased antibacterial activity [28].

The majority of VMEs occurred for β-lactams and could be tentatively attributed to an overexpression of chromosomal genes (bla_{SHV} in *K. pneumoniae*, bla_{OXY} in *K. oxytoca* and bla_{HugA} in *P. vulgaris*), which addresses the limits of WGS with regards to the levels of expression of ARGs. However, when the increased gene expression is due to gene multiplication, the use of long-read sequencing can possibly resolve this issue [29]. Otherwise, we need more data linking mutations to the expression of ARGs and mutations affecting the expression of intrinsic genes such as $bla_{AmpC,}$ or to use naive methods such as machine learning-based methods [15,30]. To the best of our knowledge, the quantification of the expression of genes (e.g. via transcriptomics) for inferring antibiotic susceptibility has not been tested for Enterobacterales. Hence for these species, the inference of some β-lactams may not be reliable and shall not be attempted. Finally, the *E. coli* strain 53, which produced a CMY-42 and an OXA-181 was resistant to cefepime. CMY-42 is a derivative of CMY-2 with a higher activity on cefotaxime and ceftazidime, but its activity on cefepime remains unclear [31]. Of note, the chromosomal bla_{AmpC} of the strain was wild type (data not shown). The decreased susceptibility to cefepime of *E. coli* 53 could then be explained by the combination of the CMY-42 and OXA-181, the latter having a moderate activity against cefepime [32]).

Our study has limitations. We analysed a limited number of strains per species, and our panel may not be representative of the every-day situation in clinical laboratories. We analysed various resistance profiles for *E. coli* and *K. pneumoniae*, while more wild-type strains were included for other species such as *C. koseri* and *P. vulgaris*. The predictive values of WGS for inferring susceptibility depend on the local epidemiology and were not calculated here. Also, we only considered the antibiotics given in probabilistic regimen of sepsis and not others such as cotrimoxazole or tetracycline which could be used after de-escalation. Finally, we used the disc diffusion method as reference for antibiotic susceptibility testing while it may not be optimal for some antibiotics such as piperacillin-tazobactam. We also acknowledge that the cefepime and ciprofloxacin cut-offs have changed from EUCAST v6.0 and may have lead us to false susceptible results by disc diffusion.

Clinical metagenomics is expected to reach diagnostic laboratories in the coming years. In the context of sepsis, clinical metagenomics has the potential to decrease the time to tailored and definitive antibiotic therapies, potentially providing higher chances of cure or a better outcome. Hence, we chose to consider only a limited set of antibiotics—those used in the context of probabilistic treatment of sepsis. We also chose to avoid inference attempts when available data would not support a strong prediction (essentially due to gene expression unpredictability), leaving some uncertainty for some species−antibiotic combinations. From this clinical perspective, we observed very good performances for ceftazidime (in non-AmpC producing strains), cefepime (all strains), fluoroquinolones and aminoglycosides, with rare VMEs in our set. Our results support that clinical metagenomics could allow a reliable antibiotic susceptibility prediction for relevant antibiotics in the sepsis context.

Transparency declaration

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Appendix A. Supplementary data

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