Evaluation study of using ampicillin susceptibility to predict imipenem susceptibility of E. faecalis and E. faecium

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Research article

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Abstract

Objective: To evaluate ampicillin to predict activity of Enterococcus faecalis and Enterococcus faecium to imipenem. Methods: A total of 127 non-duplicated strains of Enterococcus faecalis and 124 strains of Enterococcus faecium were collected from 23 hospitals in China. The antimicrobial susceptibility testing was determinated using broth microdilution and disk diffusion. Results: For all E. faecalis , when using penicillin/ampicillin results to predict susceptibility to imipenem (called as penicillin-imipenem prediction mode and amipicillin-imipenem mode), the categorical agreement (CA) and major error (ME) rate was 88.9%/95.3% and 6.3%/0%, whereas it was 89.7%/96.8% and 8.7%/1.6%, when using that results of disk diffusion, respectively. No very major error (VME) rate was founded for both prediction modes. For penicillin susceptible, ampicillin susceptible E. faecalis , the CA rate of ampicillin-imipenem prediction mode based on results from broth microdilution and disk diffusion to was both 100%, and neither was founded with VME or ME rate. For penicillin resistant, ampicillin susceptible E. faecalis , the CA rate of ampicillin-imipenem prediction mode based on results from broth microdilution and disk diffusion was 57.1% and 81.8%, respectively. And neither was founded with VME or ME rate. For penicillin resistant, ampicillin resistant E. faecalis , the CA/ME/VME rate of ampicillin-imipenem prediction mode based on results from broth microdilution and disk diffusion was 100%/0%/0% and 77.8%/22.2%/0%, respectively. For all E. faecium , the CA rate of penicillin-imipenem and ampicillin-imipenem prediction mode based on results from broth microdilution was 100% and 99.2%, and it was both 99.2% based on results from disk diffusion. ME and VME rate for all four prediction modes was 0%. For penicillin resistant, ampicillin resistant E. faecium , the CA rate was 100%, as well as penicillin susceptible, ampicillin susceptible E. faecium . None of prediction mode was found with ME or VME rate. Conclusion: For penicillin susceptible, ampicillin susceptible or penicillin resistant, ampicillin resistant E. faecalis and E. faecium , ampicillin susceptibility results of broth microdilution could accurately predict in vitro activity of imipenem. However, for penicillin resistant, ampicillin susceptible E. faecalis and E. faecium , using ampicillin results to predict imipemem susceptibility of was poorly consistent.

Background

With the wide spread use of antimicrobial agents and various invasive medical devices, infection caused by the *Enterococcus spp.* are gradually increasing, including bloodstream infection, surgical infection, urinary tract infection and abdominal infection (1,2). Antimicrobial agents selection for the treatment of *Enterococcus spp.* related infections is difficult with the characteristics of natural resistance and acquired resistance. Penicillin and ampicillin are the preferred drugs for the treatment of Enterococcus, especially *E. faecalis* related infections, because of its low adverse effects and high susceptibility. However, with the emergence of major drug resistance mechanisms such as beta-lactamase production and penicillin binding protein (PBPs) mutation, the resistance of *Enterococcus* spp. to penicillin and ampicillin increased (3,4). Additionally, imipenem has been approved for the treatment of *E. faecalis* related infections by the FDA. For *Enterococcus* spp., the clinical breakpoint of imipenem is absence in the “Performance Standards for Antimicrobial Susceptibility Testing” of CLSI, but it is clearly mentioned that

ampicillin-susceptible *E. faecalis* are predictably susceptible to imipenem. And Weinstein MP et al. also described in their study that penicillin and ampicillin susceptibility could accurately predict susceptibility to imipenem of *E. faecalis* and *E. faecium* (6,7). However, Conceicao N et al. reported that result of ampicillin susceptibility test cannot be used to predict imipenem susceptibility of penicillin-resistant and ampicillin-susceptible *E. faecalis*, which indicated that strains form different countries or regions may be different in antimicrobial susceptibility phenotype (8,9). In order to study the reliability of using ampicillin susceptibility result to predict imipenem susceptibility of *E. faecalis* and *E. faecium* isolated in China, 127 strains of *E. faecalis* and 124 strains of *E. faecium* were collected from 23 hospitals across the countries to performed with antimicrobial susceptibility tests of penicillin, ampicillin and imipenem, respectively.

Methods

Isolate collection.

A total of 127 nonduplicate isolates of *E. faecalis* and 124 nonduplicate isolates of *E. faecium* were collected from 23 hospitals across China from 2017-2018 (as one of projects by CHINET surveillance, www.chinets.com). Species identification was performed at each participating medical center and be re-confirmed in our laboratory using matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDITOF/MS; Bruker, Billerica, MA), when necessary. *E. coli* strain ATCC 25922, *S. aureus* strain ATCC25923 and *E. faecalis* strain ATCC29212 were used as the quality control strains for the antimicrobial susceptibility testing.

Antimicrobial susceptibility testing.

Penicillin, ampicillin and imipenem susceptibility testing and results interpretation for all Enterococcus isolates were performed using broth micro dilution (BMD) and disc diffusion according to the CLSI guidelines. In absence of imipenem breakpoint for *Enterococcus spp.* in the CLSI, the breakpoints from US Food and Drug Administration (FDA) (susceptible (S) $\leq$ 4, inter-mediate (I) 8, resistant (R) $\geq$ 16 mg/L for MIC testing; S $\geq$ 16, I 14-17, S $\leq$ 13 mm for disc diffusion) was referred in our study.

Evaluation index.

Evaluation index include category agreement (CA), which is the percentage of strains whose susceptibility results (suceptible, inter-mediate, resistant) of the evaluated method are consistent with the reference method. Very major error (VME) means that the evaluated method misjudges resistant strains as susceptible strains. Major error (ME) means that the evaluated method misjudges susceptible strains as resistant strains. Acceptable range: CA $\geq$ 90%, ME $\leq$ 3% and VME $\leq$ 1.5% (12).

Results

Antimicrobial susceptibility testing. For 127 strains of *E. faecalis*, the MIC range of ampicillin and imipenem was both 0.06->128 μg/mL and $MIC_{50/90}$ was 0.5/2 and 1/8 μg/mL, respectively; for 124

strains of *E. faecium*, it was 0.25->128 μg/mL and >128/>128 for both ampicillin and imipenem.

Assessment of using penicillin and ampicillin susceptibility to predict imipenem susceptibility of *E. faecalis* and *E. faecium*.

For all *E. faecalis*, when using penicillin susceptibility result of broth microdiluton to predict susceptibility to imipenem (penicillin-imipenem prediction mode), the categorical agreement (CA) and major error (ME) rate was 88.9% and 6.3%, and it was 89.7% and 8.7%, when using that result of disk diffusion, respectively. The CA and ME rate was 95.3%, 96.8% and 0%, 1.6% for ampicillin-imipenem prediction when using results from broth microdiluton and disk diffusion, respectively. No very major error (VME) rate was found for either penicillin-imipenem or ampicillin-imipenem prediction. For penicillin susceptible- and ampicillin susceptible- *E. faecalis* (107/127), the CA rate of ampicillin-imipenem prediction mode based on results from broth microdilution and disk diffusion was both 100%, and neither was founded with VME or ME rate. For penicillin resistant- and ampicillin susceptible- *E. faecalis*, the CA rate of ampicillin-imipenem prediction mode based on results from broth microdilution (14/127) and disk diffusion (11/127) was 57.1% and 81.8%, respectively. Neither was founded with VME or ME rate. For penicillin resistant- and ampicillin resistant- *E. faecalis*, the CA/ME/VME rate of ampicillin-imipenem prediction mode based on results from broth microdilution (6/127) and disk diffusion (9/127) was 100%/0%/0% and 77.8%/22.2%/0%, respectively.

For all *E. faecium*, the CA rate of penicillin-imipenem and ampicillin-imipenem prediction mode based on results from broth microdilution was 100% and 99.2%, and it was both 99.2% based on results from disk diffusion. ME and VME rate for all four prediction modes was 0%. For penicillin susceptible, ampicillin susceptible *E. faecium*, the CA rate of ampicillin-imipenem prediction mode based on results from broth microdilution (15/124) and disk diffusion (14/124) was both 100%. For penicillin resistant, ampicillin resistant *E. faecium*, the CA rate was 100% based on results from broth microdilution (108/124) and disk diffusion (109/124). Only one E. faecium isolate was resistant to penicillin, imipenem and susceptible to ampicillin. None of prediction mode was found with ME or VME rate.

Discussion

Enterococcus spp. is one of the most common opportunistic pathogens caused nosocomial infection after S. aureus in all gram-positive bacteria (13). It accounted for 8.42% (16043/190610) among all clinical isolates and ranked seventh, according to the results from the China antimicrobial resistance surveillance network (CHINET) in 2017. Severe infection caused by the Enterococcus would be a danger to the patient's life, and combination therapies of penicillin or ampicillin and aminoglycoside are usually used for the treatment of Enterococcus related infections (14). In addition, studies also shown that imipenem is certain clinically effective against Enterococcus infection (15,16). The determination standard of imipenem susceptibility testing against the Enterococcus is absence at present, and CLSI only mentioned that the susceptibility result of ampicillin can be used to predict the susceptibility to

imipenem of *E. faecalis*. According to study, whether ampicillin susceptibility can accurately predict imipenem susceptibility of *E. faecalis* is associated with the susceptibility result of penicillin.

Grouped based on penicillin susceptibility results, for *E. faecalis* with opposite susceptibility results of penicillin and ampicillin, the CA, VME and ME of using ampicillin susceptibility to predict imipenem susceptibility was different. For example, for penicillin-resistant, ampicillin-susceptible *E. faecalis*, the CA of using ampicillin susceptibility to predict imipenem susceptibility tested by broth microdilution and disk dilution was 57.1% and 81.8%, respectively, and imipenem inter-mediate isolates accounted for 42.9% and 18.2%. For penicillin-resistant, ampicillin-resistant or penicillin-susceptible, ampicillin-susceptible *E. faecalis*, ampicillin susceptibility of broth microdilution could both accurately predict imipenem susceptibility (CA/VME/ME=100%/0%/0%). Conceicao N et al. also reported that *E. faecalis* isolates susceptible to both penicillin and ampicillin were also susceptible to imipemem tested by broth microdilution, and it was consistent with our study (8). It should be noted that for *E. faecalis* resistant to both penicillin and ampicillin tested by disc diffusion, ampicillin susceptibility could not be used to predict imipenem susceptibility (the CA and ME of ampicillin-impenem prediction was 77.8% and 22.2%). In recent years, penicillin-resistant, ampicillin-susceptible E. faecalis has been reported frequently (17,18). As Metzidie et al. reported, the detection rate was 31.4%, higher than that in our study (11% tested by broth microdilution and 8.7% tested by disc diffusion), and most strains was resistant to imipenem (18). Weinstein MP et al. found that the CA of using penicillin susceptibility to predict imipenem susceptibility tested by broth microdilution was 95.2%; and the CA of using ampicillin susceptibility to predict imipenem susceptibility tested by disk diffusion was 99.8% (ME=0.2%) (7). The result ignores penicillin-resistant, ampicillin-susceptibile *E. faecalis*, leading us to mistakenly believe that ampicillin susceptibility can predict imipenem susceptibility of all *E. faecalis* with different susceptibility phenotypes.

In our study, the penicillin-resistant, ampicillin-susceptible *E. faecium* strains are rare (1/124), and for penicillin-susceptible, ampicillin-susceptible or penicillin-resistant, ampicillin-resistant *E. faecium*, the CA of using ampicillin susceptibility to predict imipenem susceptibility of was both 100%. And for all *E. faecium*, it was 99.2% tested by either broth microdilution or disk diffusion, which was both 98% in the study of Weinstein MP et al. Based on this, we believe that ampicillin susceptibility can predict imipenem susceptibility of *E. faecalis*, except for the rare penicillin-resistant, ampicillin-susceptible isolates.

This study evaluated the feasibility of using penicillin and ampicillin susceptibility to predict imipenem susceptibility of *E. faecalis* and *E. faecium*. In conclusion, the susceptibility result of ampicillin is superior to that of penicillin to predict imipenem in vitro activity for *E. faecalis*. And susceptibility of both of them can be used as a predictor of imipenem in vitro activity for *E. faecium*. However, it should be emphasized that ampicillin susceptibility cannot be used to predict imipenem susceptibility of *E. faecalis* and *E. faecium* with penicillin-resistant, ampicillin-susceptible susceptibility phenotypes.

Abbreviations

CA, Category agreement; ME, Major error; VME, Very major error; BMD, Broth micro dilution; CLSI, Clinical and Laboratory Standard Intstitute

Declarations

Acknowledgment

Not Applicable

Authors' contributionsLG and DY contributed to study conception, data acquisition, analysis and interpretation, manuscript drafting and revising; YG, YZ, QS, YY contributed to data analysis; DZ and FH contributed to study conception and design, data acquisition, analysis and interpretation, manuscript revising. All authors read and approved the final manuscript.

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Availability of data and materials

Except for participant identifying information, all data generated or analysed during this study are included in this published article.

Ethics approval and consent to participate

The study was approved by ethics review boards at HuashanHospital, Fudan University (KY2018-218,01, on Sep 1 2017).

All participants provided written informed consent to participate in the study.

Consent for publication

All participants provided written informed consent for their data to be disseminated, including publication in peer-reviewed journals.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table1. Evaluation of using ampicillin or penicillin susceptibility to predict imipenem in vitro activity of *E. faecalis* and *E. faecium,* tested by broth microdilution and disk diffusion.

Organism		broth microdilution(BMD)						disk diffusion(DD)					
Group of isolates		Imipenem susceptibility			Evaluation index of using penicillin or ampicillin as predictor			Imipenem susceptibility			Evaluation index of using penicillin or ampicillin as predictor		
		S	I	R	CA%	No. (%) of VMEs	No. (%) of MEs	S	I	R	CA%	No. (%) of VMEs	No. (%) of MEs
E. faecalis	Penicillin[a]	107	0	6	88.9	0	8(6.3)	107	0	7	89.7	0	11(8.7)
	Ampicillin[b]	115	0	6	95.3	0	0	116	0	7	96.8	0	2(1.6)
	Penicillin-S & Ampicillin-S	107	0	0	100	0	0	107	0	0	100	0	0
	Penicillin-R & Ampicillin-S	8	6	0	57.1	0	0	9	2	0	81.8	0	0
	Penicillin-R & Ampicillin-R	0	0	6	100	0	0	2	0	7	77.8	0	2(22.2)
E. faecium	Penicillin[c]	15	0	109	100	0	0	14	0	109	99.2	0	0
	Ampicillin[d]	15	0	108	99.2	1(0.8)	0	14	0	109	99.2	0	0
	Penicillin-S & Ampicillin-S	15	0	0	100	0	0	14	0	0	100	0	0
	Penicillin-R & Ampicillin-S	0	0	1	0	100	0	0	0	1	0	100	0
	Penicillin-R & Ampicillin-R	0	0	108	100	0	0	0	0	109	100	0	0

[a]penicillin-susceptible strains of *E. faecalis*; [b]ampicillin-susceptible strains of *E. faecalis*; [c]penicillin-susceptible strains of *E. faecium* to penicillin; [d]ampicillin-susceptible strains of *E. faecium*

Figures

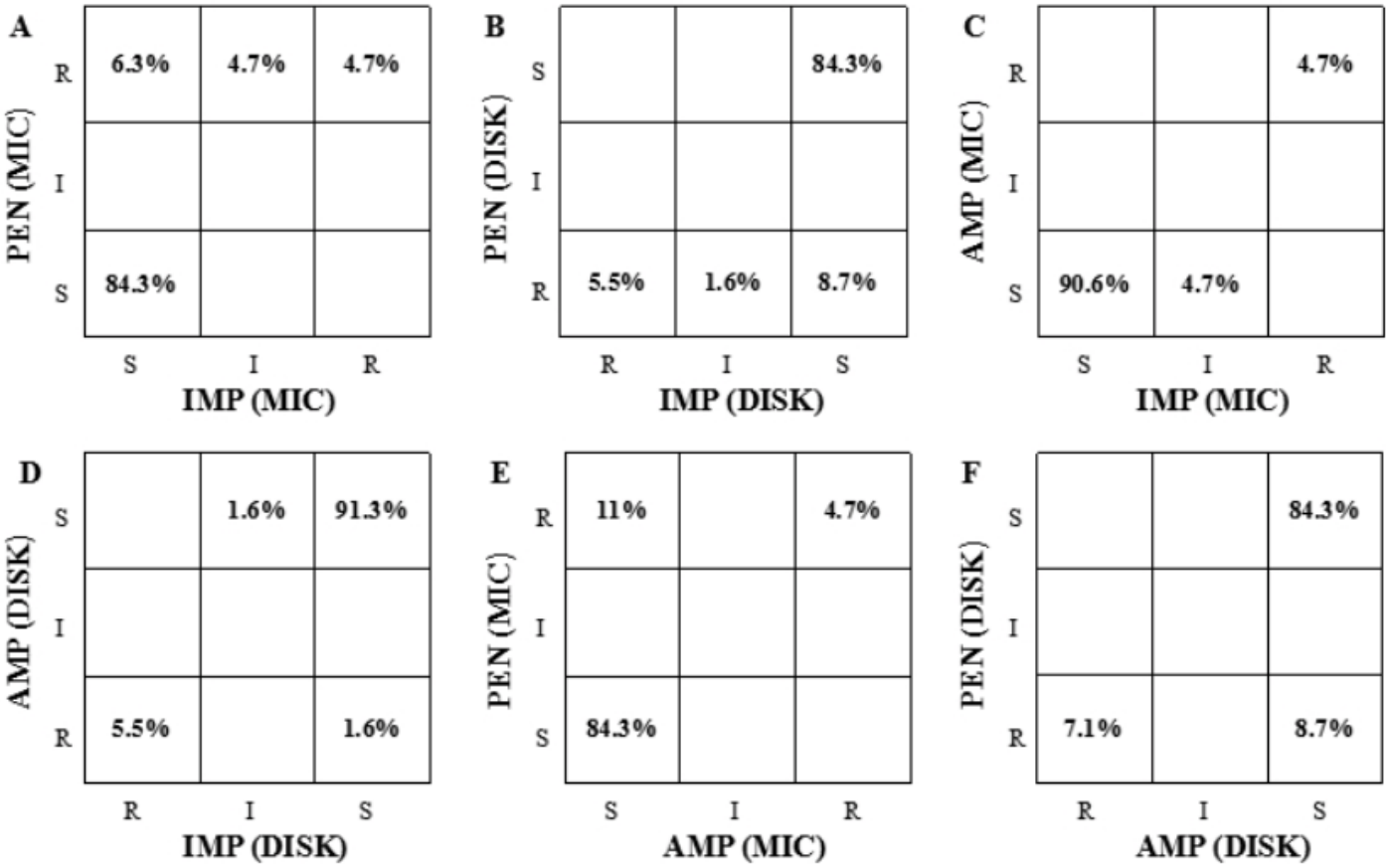

Figure 1

Correlation data for in vitro susceptibility of E. faecalis: imipenem versus penicillin by BMD (A), imipenem versus penicillin by DD (B), imipenem versus ampicillin by BMD (C), and imipenem versus ampicillin by DD (D), penicillin versus ampicillin by BMD (E), penicillin versus ampicillin by DD (F).

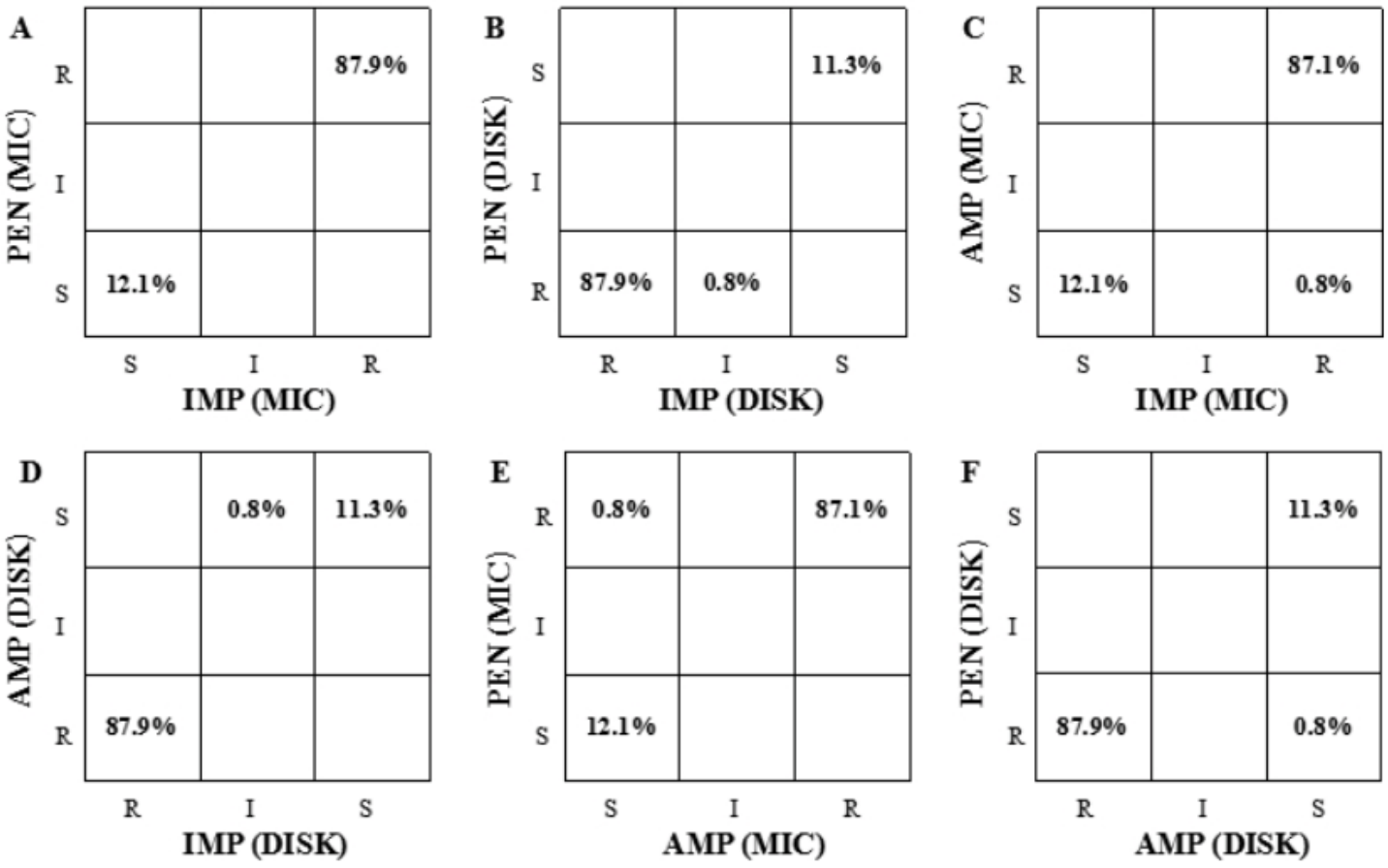

Figure 2

Correlation data for in vitro susceptibility testing of E. faecium: imipenem versus penicillin by BMD (A), imipenem versus penicillin by DD (B), imipenem versus ampicillin by BMD (C), and imipenem versus ampicillin by DD (D),penicillin versus ampicillin by BMD (E),penicillin versus ampicillin by DD (F).