CLSI – EUCAST: COMPARISON OF ANTIBIOTIC-SUSCEPTIBILITY PROFILE OF *ENTEROBACTERIACEAE* OF ANIMAL ORIGIN ACCORDING TO THE STANDARDS

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Antimicrobial resistance is a relevant "One Health" issue that shows the need of comparison of isolates of different origins. In this way, guidelines for antimicrobial-resistance evaluation in animals are relevant in relation to human sources. This work aims to compare antimicrobial-resistance results of animal isolates considering CLSI and EUCAST guidelines. The comparison shows considerable differences in the results, which include antibiotics used as primary options in hospital infections. EUCAST showed the higher number of samples with resistance profiles than CLSI that indicates a more efficient scenario to the EUCAST to screen antibiotic-resistant bacteria. EUCAST was more consonant to the expected phenotype for ESBL producers, with higher index of resistance to oxyimino-beta-lactam antibiotics. The study shows that there are differences in the interpretative results using different guidelines, where the susceptibility test results concerning *Enterobacteriaceae* of animal origin are not always coincident in CLSI and EUCAST. EUCAST has proved to be the most reliable alternative for profile screening of antibiotic resistance, when compared to CLSI. We might say the same with respect to the ESBL-producing *Enterobacteriaceae*, in which EUCAST has proved to be more efficient about the demonstration of expected resistance profiles for the ESBL producers. These differences show that guideline selection might influence the therapeutic option.

Keywords: CLSI, EUCAST, *Enterobacteriaceae*, susceptibility testing, animal

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Introduction

Antibiotic resistance is a problem that has been going worse and worse in the past decades. Therefore, detection and monitoring of the resistant microorganisms and their mechanisms of resistance is of great importance. Antibiotic susceptibility is evaluated using directions from notable institutions that are recognized by the scientific community. The current leading institutions that supply these standards for antibiotic susceptibility evaluation are the Clinical and Laboratory Standards Institute (CLSI) from North America and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) from Europe. The CLSI guidelines for Antibiotic Susceptibility Tests (ASTs) were mostly used in the world for many years, given its credibility (the Institute began as a National Committee – NCCLS – almost 50 years ago) and were recognized in United States of America by Food and Drug Administration. Laboratories need to pay an annual signature to have access to the current version that is updated every year [1, 2]. The EUCAST has been recognized at European level; it has been only 20 years and has emerged from the unification of several European reference institutions, which justifies its credibility and accessibility regarding the microbiology laboratories, once its content is available for free [3, 4].

At present, there are no reference standards for AST interpretation concerning microorganisms of animal origin in EUCAST. CLSI produces annually the CLSI VET, which establishes criteria for samples of animal origin, that is widely used in the United States [5]. However, for AST of bacteria of animal origin, whether it is for animal prescription or for scientific research purposes, in European reality, as for other countries, the human standard references of CLSI and EUCAST are the most used [6–11].

Several studies have analyzed the differences and similarities of the results obtained by the guidelines of both institutions regarding fungi and Gram-positive and Gram-negative bacteria, yet the data related to the comparative evaluation of results of *Enterobacteriaceae* from animal production are scarce and of great importance to the comprehension of the differences that might exist between these interpretations and their impact on resistance to antibiotics of the samples of animal origin, which much concerns human health when analyzed by the perspective of One Health [1, 12–18].

Materials and Methods

A range of 309 *Enterobacteriaceae* isolated from samples of healthy bovine feces, of the production environment (manure, ration, and water consumed by the

animals), and of other animal products (milk), were submitted to AST evaluation by the agar diffusion method to 25 antimicrobials present on the interpretations by this method in CLSI [19] and EUCAST [20]. Nitrofurantoin and fosfomycin were analyzed only for *Escherichia coli* (266 strains). The samples from Brazilian animal productions were obtained in April 2014. The obtained results were classified as resistant, intermediate, or susceptible according to each institution. The data were inserted into the SSPS software version 25 (IBM, USA), in which the existent similarity between the obtained results by CLSI and EUCAST criteria was interpreted. In addition, the "kappa" coefficient from the relationship between results was analyzed to determine the relationship between both the results without the influence of the statistic random. It is interpreted between "perfect agreement" and "poor relationship."

Results

The 25 antimicrobials analyzed showed differences varying between 99.4% and 67.4%. Those that showed high percentage of similarity were: sulfamethoxazole + trimethoprim (99.4%), meropenem (98.4%), amoxicillin (97.7%), imipenem (97.7%), netilmicin (97.6%), and fosfomycin and amikacin (96.7%). Studies performed using the 2016 version of CLSI and EUCAST also showed percentages of similarity above 95% for sulfamethoxazole + trimethoprim and meropenem [1]. Some antimicrobials showed low similarity values among the obtained results: ceftaroline (67.4%), piperacillin + tazobactam (73%), aztreonam (76.3%), piperacillin (76.4%), and ciprofloxacin (76.6%). Table I shows similarity values and "kappa" coefficient analysis for the 25 antimicrobials analyzed.

The "kappa" coefficient analysis results show that the results of no antimicrobial are in perfect agreement once no "kappa" coefficient 1 was obtained. The best "kappa" coefficient results were classified as almost perfect regarding amoxicillin – k of 0.952 (95% CI = 0.917, 0.987), cefoxitin – k of 0.844 (95% CI = 0.757, 0.930), amikacin – k of 0.819 (95% CI = 0.708, 0.929), sulfamethoxazole + trimethoprim – k of 0.987 (95% CI = 0.969, 1.005), and chloramphenicol – k of 0.810 (95% CI = 0.730, 0.891). The classification "substantial" for the coefficient "kappa" was the result that showed the biggest antimicrobials number, as amoxicillin + clavulanic acid – k of 0.609 (95% CI = 0.517, 0.700), piperacillin – k of 0.612 (95% CI = 0.524, 0.700), cefuroxime – k of 0.676 (95% CI = 0.594, 0.758), cefotaxime k of 0.717 (95% CI = 0.632, 0.803), cefepime – k of 0.741 (95% CI = 0.660, 0.822), meropenem – k of 0.661 (95% CI = 0.366, 0.956), imipenem – k of 0.749 (95% CI = 0.565, 0.933), doripenem – k of 0.717 (95% CI = 0.594, 0.840), levofloxacin – k of 0.672 (95% CI = 0.579, 0.765), gentamicin – k of 0.766

Table I. *Enterobacteriaceae* antibiotic susceptibility according to CLSI (2017) and EUCAST (2017) guidelines, similarities, and "kappa" coefficient analysis

Antibiotic	CLSI			EUCAST			Similarity (%)	Kappa (k) coefficient	Cohen's interpretation	95% CI	
	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)					
Amoxicillin	63.1	2.3	34.6	63.1	0.0	36.9	97.7	0.952	Almost perfect	0.917	0.987
Amoxicillin + clavulanic acid	20.4	18.4	61.2	20.4	0.0	79.6	81.6	0.609	Substantial	0.517	0.700
Piperacillin	46.7	18.2	35.1	50.4	9.9	39.7	76.4	0.612	Substantial	0.524	0.700
Piperacillin + tazobactam	7.1	22.0	71.0	6.2	10.4	83.4	73.0	0.292	Fair	0.145	0.439
Cefuroxime	24.6	17.3	58.1	34.3	0.0	65.7	82.7	0.676	Substantial	0.594	0.758
Cefotaxime	32.2	10.6	57.1	29.0	4.1	66.9	85.3	0.717	Substantial	0.632	0.803
Ceftazidime	10.2	5.3	84.4	16.8	13.5	69.7	78.3	0.439	Moderate	0.306	0.573
Cefepima	11.0	19.1	69.9	16.5	7.8	75.7	88.7	0.741	Substantial	0.660	0.822
Ceftaroline	28.7	7.4	64.0	61.2	0.0	38.8	67.4	0.435	Moderate	0.336	0.534
Aztreonam	12.0	5.5	82.5	17.5	18.2	64.3	76.3	0.460	Moderate	0.352	0.568
Cefoxitin	11.0	2.6	86.4	14.9	0.0	85.1	96.1	0.844	Almost perfect	0.757	0.930
Ertapenem	3.1	2.4	94.4	5.6	6.6	87.8	91.0	0.459	Moderate	0.261	0.658
Meropenem	1.3	1.6	97.1	0.6	1.3	98.1	98.4	0.661	Substantial	0.366	0.956
Imipenem	1.3	4.2	94.4	0.7	3.3	96.1	97.7	0.749	Substantial	0.565	0.933
Doripenem	2.1	8.3	89.6	3.5	12.1	84.4	93.4	0.717	Substantial	0.594	0.840
Ciprofloxacin	32.1	5.5	62.3	46.1	9.4	44.5	76.6	0.589	Moderate	0.506	0.672
Levofloxacin	32.5	3.8	63.7	37.3	13.7	49.1	81.6	0.672	Substantial	0.579	0.765
Gentamicin	5.5	4.2	90.2	7.2	5.9	87.0	95.1	0.766	Substantial	0.651	0.882
Tobramycin	2.9	3.6	93.5	3.6	7.5	88.9	94.8	0.684	Substantial	0.533	0.835
Amikacin	1.6	6.5	91.9	1.6	9.8	88.6	96.7	0.819	Almost perfect	0.708	0.929
Netilmicin	2.4	0.9	96.7	2.8	2.4	94.8	97.6	0.713	Substantial	0.465	0.962
Nitrofurantoin[a]	4.4	15.0	80.6	0.5	0.0	99.5	81.1	0.043	Slight	−0.227	0.314
Sulfamethoxazole + trimethoprim	42.5	1.3	56.2	42.5	0.6	56.8	99.4	0.987	Almost perfect	0.969	1.005
Chloramphenicol	14.7	6.5	78.8	20.5	0.0	79.5	93.5	0.810	Almost perfect	0.730	0.891
Fosfomycin[a]	1.3	0.9	97.8	4.0	0.0	96.0	97.3	0.561	Moderate	0.215	0.908

Note: CLSI: Clinical and Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing; R: resistant; S: susceptible; I: intermediate; CI: confidence interval.

[a]Only for *Escherichia coli.*

(95% CI = 0.651, 0.882), tobramycin – k of 0.684 (95% CI = 0.533, 0.835), and netilmicin – k of 0.713 (95% CI = 0.465, 0.962). The results classified as "moderate" are for ceftazidime – k of 0.439 (95% CI = 0.306, 0.573), ceftaroline – k of 0.435 (95% CI = 0.336, 0.534), aztreonam – k of 0.460 (95% CI = 0.352, 0.568), ertapenem – k of 0.459 (95% CI = 0.261, 0.658), ciprofloxacin – k of 0.589 (95% CI = 0.506, 0.672), and fosfomycin – k of 0.561 (95% CI = 0.215, 0.908). Coefficient "kappa" results that showed the lowest classification, as "fair" and "slight," respectively, were piperacillin + tazobactam – k of 0.292 (95% CI = 0.145, 0.439) and nitrofurantoin – k of 0.043 (95% CI = −0.227, 0.314).

When we compare the number of samples classified as resistant regarding both the standards, we discover a scenario in which few results are coincident. Four antibiotics (amoxicillin, amoxicillin + clavulanic acid, amikacin, and sulfamethoxazole + trimethoprim) showed similar results to the resistance profile concerning the susceptibility evaluation. In regard to five antibiotics (piperacillin + tazobactam, cefotaxime, meropenem, imipenem, and nitrofurantoin), the CLSI criteria showed more samples considered as resistant, when compared to EUCAST, with emphasis on nitrofurantoin (CLSI – 4.4% and EUCAST – 0.5%). The other 16 antibiotics (piperacillin, cefuroxime, ceftazidime, cefepime, ceftaroline, aztreonam, ertapenem, doripenem, ciprofloxacin, levofloxacin, gentamicin, tobramycin, netilmicin, chloramphenicol, and fosfomycin) showed higher number of resistant samples for EUCAST, presenting in some cases high discrepancy, as for ceftaroline (EUCAST – 61.2% and CSLI – 28.7%), ciprofloxacin (EUCAST – 46.1% and CLSI – 32.1%), and cefuroxime (EUCAST – 34.3% and CLSI – 24.6%). Figure 1 presents the resistance profile for all 25 antibiotics, concerning CLSI and EUCAST.

Among the 309 *Enterobacteriaceae* samples analyzed, 77 were extended-spectrum beta-lactamase producers (ESBL), and when we look at their susceptibility profile to third-, fourth-, and fifth-generation cephalosporins and to monobactams, it is observed that there is a higher number of samples classified as resistant (except for cefotaxime) in the EUCAST interpretation. This difference is very clear for ceftazidime (EUCAST – 29.1% and CLSI – 12.7%), cefepime (EUCAST – 53.2% and CLSI – 33.8%), and aztreonam (EUCAST – 29.9% and CLSI – 15.6%), once the number of isolates with resistance profiles is superior to the obtained by CLSI, and it might be the reason that masks the positive results of AST of possible ESBL producers that do not show the typical synergism. This resistance profile difference might show the hydrolytic strength for low-level beta-lactamases, when CSLI is utilized. Figure 2 illustrates the comparative profiles concerning the 77 ESBL-producing *Enterobacteriaceae* to the third-, fourth-, and fifth-generation cephalosporins and to monobactams.
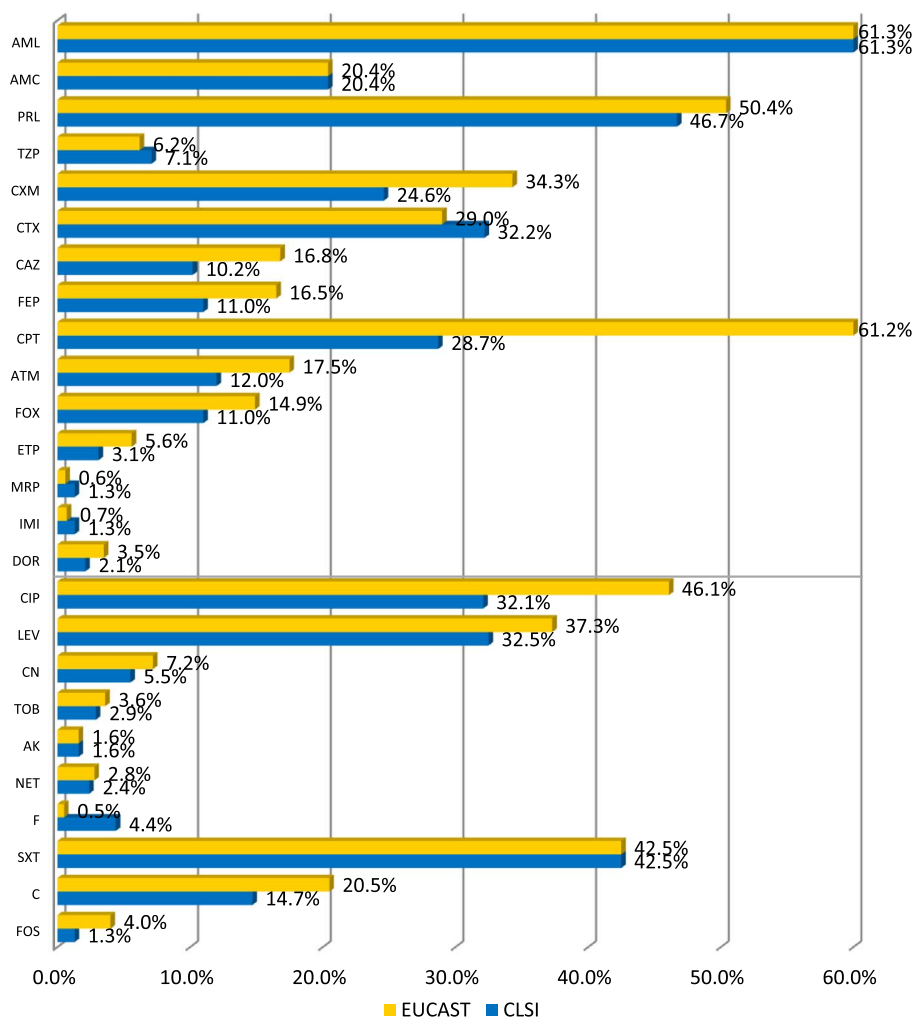
Figure 1. Antibiotic resistance profile of *Enterobacteriaceae* strains according to CLSI (2017) and EUCAST (2017) standards. AML: amoxicillin; AMC: amoxicillin + clavulanic acid; PRL: piperacillin; TZP: piperacillin + tazobactam; CXM: cefuroxime; CTX: cefotaxime; CAZ: ceftazidime; FEP: cefepime; CPT: ceftaroline; ATM: aztreonam; FOX: cefoxitin; ETP: ertapenem; MRP: meropenem; IMI: imipenem; DOR: doripenem; CIP: ciprofloxacin; LEV: levofloxacin; CN: gentamycin; TOB: tobramycin; AK: amikacin; NET: netilmicin; F: nitrofurantoin; SXT: sulfamethoxazole + trimetoprim; C: chloramphenicol; FOS: fosfomycin
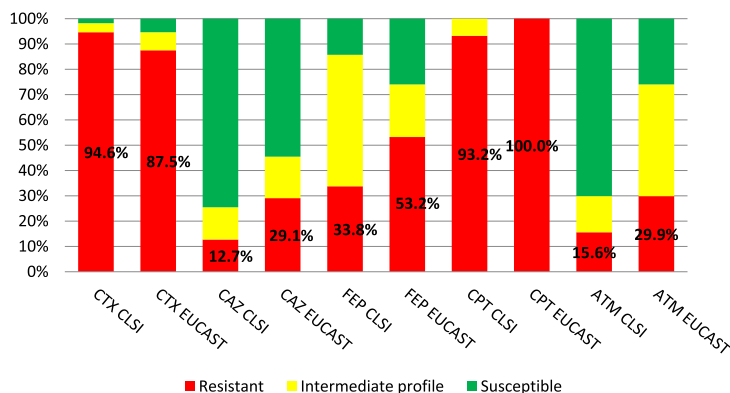
Figure 2. Susceptibility profile of ESBL-producing *Enterobacteriaceae* to third-, fourth-, and fifth-generation cephalosporins and to monobactams regarding CLSI (2017) and EUCAST (2017) standards. CTX: cefotaxime; CAZ: ceftazidime; FEP: cefepime; CPT: ceftaroline; ATM: aztreonam

Discussion

The results showed significant differences about the profile of resistance of *Enterobacteriaceae* of animal origin resistance when comparing CLSI and EUCAST standards. Some evaluated antibiotics have obtained high similarity according to Cohen's classification, such as amoxicillin, cefoxitin, amikacin, sulfamethoxazole + trimethoprim, and chloramphenicol, classified as "almost perfect." Also showing good similarity, we can find amoxicillin + clavulanic acid, piperacillin, cefuroxime, cefotaxime, cefepime, meropenem, imipenem, doripenem, levofloxacin, gentamicin, tobramycin, and netilmicin. However, six antibiotics showed similar results classified only as "moderate," such as ceftazidime, ceftaroline, aztreonam, ertapenem, ciprofloxacin, and fosfomycin, which are the antibiotics used as first options in hospital infections (ceftazidime and aztreonam) and as alternative for infections caused by ESBL producers (ciprofloxacin and fosfomycin). These differences show that the selection of the standard might influence the therapeutic option [21, 22].

EUCAST showed the higher number of samples with resistance profiles more prominent than CLSI. This fact indicates a scenario in which EUCAST shows more efficient with respect to screening of the antibiotics resistance profile, revealing a higher susceptibility in animal samples, when compared to CLSI, given that for several antibiotics, EUCAST presented resistant samples, whereas in the CLSI evaluation, those would be classified as susceptible or intermediate.

With regard to the ESBL producers, the EUCAST results have proved to be more consonant to the expected phenotype for the *Enterobacteriaceae* that

produces these enzymes, that is, higher index of resistance to third-, fourth-, and fifth-generation cephalosporins and to aztreonam. A study performed with human clinical samples has demonstrated that EUCAST is more efficient to the ESBL detection [23].

Conclusions

The similarity of the susceptibility test results concerning *Enterobacteriaceae* of animal origin is not always coincident in CLSI and EUCAST, due to different interpretations on the minimum inhibitory concentrations. Although EUCAST has proved to be the most reliable alternative for profile screening of resistance to antibiotics, once it has shown more efficient concerning detection of resistance profiles by the agar diffusion method, when compared to CLSI. We might say the same with respect to the ESBL-producing *Enterobacteriaceae*, in which EUCAST has proved to be more efficient about the demonstration of expected resistance profiles for the producers of these enzymes.

Acknowledgements

Conflict of Interest

The authors declare no competing interests.

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