BRITISH JOURNAL OF BIOMEDICAL SCIENCE, 2018
VOL. 75, NO. 1, 24–29
https://doi.org/10.1080/09674845.2017.1392736



# Assessment of the comparability of CLSI, EUCAST and Stokes antimicrobial susceptibility profiles for Escherichia coli uropathogenic isolates

C O'Halloran[a], N Walsh[a], MC O'Grady[b], L Barry[b], C Hooton[b], GD Corcoran[b] and B Lucey[a]

[a]Department of Biological Sciences, Cork Institute of Technology, Bishopstown, Cork, Ireland; [b]Department of Clinical Microbiology, Cork University Hospital, Wilton, Cork, Ireland

**ABSTRACT**

**Background:** As many clinical laboratories convert between Stokes, Clinical and Laboratory Standards Institute (CLSI) and European Committee for Antimicrobial Susceptibility Testing (EUCAST) methods, the problem of comparing differently derived sets of antimicrobial susceptibility testing (AST) data with each other arises, owing to a scarcity of knowledge of inter-method comparability. The purpose of the current study was to determine the comparability of CLSI, EUCAST and Stokes AST methods for determining susceptibility of uropathogenic *Escherichia coli* to ampicillin, amoxicillin-clavulanate, trimethoprim, cephradine/cephalexin, ciprofloxacin and nitrofurantoin.

**Methods:** A total of 100 *E. coli* isolates were obtained from boric acid urine samples from patients attending GP surgeries. For EUCAST and CLSI, the Kirby-Bauer disc diffusion method was used and results interpreted using the respective breakpoint guidelines. For the Stokes method, direct susceptibility testing was performed on the urine samples.

**Results:** The lowest levels of agreement were for amoxicillin-clavulanate (60%) and ciprofloxacin (89%) between the three AST methods, when using 2017 interpretive guidelines for CLSI and EUCAST. A comparison of EUCAST and CLSI without Stokes showed 82% agreement for amoxicillin-clavulanate and 94% agreement for ciprofloxacin. Discrepancies were compounded by varying breakpoint susceptibility guidelines issued during the period 2011–2017, and through the inclusion of a definition of intermediate susceptibility in some cases.

**Conclusions:** Our data indicate that the discrepancies generated through using different AST methods and different interpretive guidelines may result in confusion and inaccuracy when prescribing treatment for urinary tract infection.

**ARTICLE HISTORY**
Received 8 May 2017
Accepted 11 August 2017

**KEYWORDS**
EUCAST; CLSI; stokes; antimicrobial susceptibility testing; comparability assessment; *Escherichia coli*

## Introduction

Urinary Tract Infections (UTI) are an important health concern, with approximately 50% of women developing a symptomatic UTI at some stage in their lives [1]. They are also among the most commonly diagnosed infections in outpatient settings [2] and their bacteriology is predictable, with 70–95% of infections being caused by uropathogenic *Escherichia coli* (UPEC) [3]. In Europe, UTI alone account for 7% of total antibiotic usage [4]. The Kirby-Bauer method has traditionally been used for the purpose of generating an antimicrobial susceptibility profile for uropathogens using standardised inocula [5,6]. An alternative method, Direct Antimicrobial Testing, uses the specimen itself as the source of the inoculum for Antimicrobial Susceptibility Testing (AST). This Stokes susceptibility testing method was commonly used historically to determine the antimicrobial susceptibility profile of uropathogens. The advantage of this method lies in the fact that antibiotic treatment can be chosen earlier for the patient as the micro-organisms do not need time-consuming culturing and isolation before AST [7]. However, the Stokes Method lacks standardisation and associated reproducibility [8], and has been discontinued in many clinical laboratories.

More recently, the methods and interpretive guidelines of the Clinical and Laboratory Standards Institute (CLSI, Wayne, PA, USA) and the European Committee for Antimicrobial Susceptibility Testing (EUCAST) have been implemented widely for AST investigation of UTI and other infections. In response to changes in Minimum Inhibitory Concentration (MIC) distributions, pharmaco-dynamics, pharmaco-kinetics and the results of clinical trials, the CLSI releases updated breakpoints annually [9]. The use of CLSI guidelines in clinical labs in Europe has decreased in recent years, being steadily replaced by EUCAST [10]. This may have resulted in some confusion for clinicians as switching between methods has been accompanied by differing interpretive guidelines, including the insertion or removal of an intermediate susceptibility definition for certain antimicrobial agents. Dosages, pharmacokinetics, resistance mechanisms, MIC

**CONTACT** B Lucey Brigid.lucey@cit.ie

distributions, zone diameter distributions, pharmaco-dynamics, and epidemiological cut-off values are used in the breakpoint-setting process [11]. EUCAST has published updated guidelines annually since 2009, and in general, tends to recommend lower MIC breakpoints defining resistance than CLSI [12].

The purpose of the current study was to determine the comparability of CLSI, EUCAST and Stokes methods used when completing AST for urinary tract isolates or uropathogens. AST results determine optimal specific treatment for individual patients, guide empirical antibiotic therapy through incorporation into local antibiotic guidelines, and, occasionally form the basis for formulary decisions [13]. The comparability of CLSI, EUCAST and Stokes AST methods when assessing susceptibility levels of uropathogens to oral antimicrobial agents used in treating UTI has not yet been fully determined.

## Materials and methods

During January and February 2017, a collection of boric acid-treated specimens of urine submitted to the Cork University Hospital laboratory for urinalysis were investigated, from patients attending GP surgeries. The urine was used for cultivation and for direct susceptibility testing. In all, 100 *E. coli* isolates were obtained. Each of these strains was isolated from a sample which had a white blood cell count of ≥100/cm$^2$ and a bacterial count exceeding 10,000 colony forming units per mL. All isolates were identified as *E. coli* using Matrix Assisted Laser Desorption/Ionisation-Time of Flight (MALDI-TOF) Mass Spectrometry (Bruker Daltonics, Bremen, Germany), according to laboratory protocol.

The Modified Direct Stokes Disc-Diffusion AST method used was as described by Gosden [14]. The Stokes method is based on direct inoculation of the urine for AST onto Iso-sensitest agar (CM0471, Oxoid, Basingstoke, UK). This medium was prepared according to the manufacturer's instructions, and poured to a volume of exactly 25 mL per agar plate. Antimicrobial discs were applied using a dispenser and the plates were incubated at 37 °C for 18–24 h. The following concentrations of antimicrobial agents (supplied by Oxoid) were used: ampicillin (AMP 25 µg), amoxicillin-clavulanate (AMC 30 µg), nitrofurantoin (F 200 µg), cephradine (CE 30 µg), ciprofloxacin (CIP 5 µg) and trimethoprim (W 5 µg). American Type Culture Collection (ATCC) *E. coli* 25922 was used as the control strain for this test method. Zones were interpreted as sensitive if the zone radius was equal to, greater than, or not ≤3 mm smaller than the control; intermediate with a zone radius ≥2 mm from the edge of the disc but smaller than the control by >3 mm; and resistant with a zone radius of <2 mm from the edge of the disc.

An evaluation of the effect of inoculum standardisation on Stokes AST results was determined. When originally performing the Stokes method, the urine was swabbed directly onto the plate for susceptibility investigation, thereby rendering inoculum standardisation impossible, so for this part of the study, a standardised inoculum (equivalent to a 0.5 McFarland turbidity standard) was prepared in sterile 0.85% NaCl for each isolate from the original urine sample. Kirby Bauer disc-diffusion was conducted, for which purpose the *E. coli* isolates were stored on nutrient agar slopes and were sub-cultured to MacConkey agar (LAB030) and incubated at 37 °C for 18–24 h before AST was completed.

Methods were conducted following CLSI [15] and EUCAST [7] guidelines. Disc-diffusion tests were performed using Mueller-Hinton agar (LAB039, LabM Ltd., Bury, UK). The medium was prepared following the manufacturer's instructions and the plates were poured using a mass balance to a volume of 25 mL. Six oral antimicrobial agents were assessed; the concentrations of the discs vary between CLSI and EUCAST, as detailed below. The surface of the Mueller-Hinton agar plate was inoculated with a suspension of the strain to be tested to the density of a McFarland 0.5 turbidity standard, using a 0.85% solution of NaCl in water (8.5 g/L). The antibiotic discs were applied manually using a needle and the plates were incubated at 37 °C for 16–20 h. Zone diameters were measured using Mitutoyo callipers (Mituyo, Kanagawa, Japan), in accordance with the respective guidelines [16,17]. Duplicate measurements were performed for result verification. This was conducted as a blind trial and no measurements were made until all testing was completed.

For the CLSI Disc Diffusion Method (2012), the antibiotic discs (Oxoid) used were ampicillin (AMP 10 µg), amoxicillin-clavulanate (AMC 30 µg), nitrofurantoin (F 300 µg), cephalexin (CL 30 µg), ciprofloxacin (CIP 5 µg) and trimethoprim (W 5 µg). *E. coli* ATCC 25922 was included as a control in this study. For the EUCAST Disc Diffusion Method (2013), the antibiotic discs used were ampicillin (AMP 10 µg), amoxicillin/clavulanic acid (AMC 30 µg), nitrofurantoin (F 100 µg), cephalexin (CL 30 µg), ciprofloxacin (CIP 5 µg) and trimethoprim (W 5 µg). *E. coli* ATCC 35218 was incorporated into this study as a control strain.

## Results

To determine the level of agreement between EUCAST (2017), CLSI (2017) and Stokes AST methods for 100 *E. coli* isolates, the AST results for each of the three methods included in the current study for the 100 isolates of *E. coli* were compared in the context of their 3 different interpretive guidelines for 6 oral antimicrobial agents tested (Table 1).

The effect of inoculum standardisation was investigated for the Stokes method, using a subset of 30 of the *E. coli* isolates. These isolates were selected for this part of the study as they had generated a discrepancy between

**Table 1.** Antimicrobial susceptibility profiles of 100 uropathogenic *E. coli* isolates for six oral antimicrobial agents.

| | EUCAST (2017) | | | CLSI (2017) | | | Modified stokes method | | |
|---|---|---|---|---|---|---|---|---|---|
| | S | I | R | S | I | R | S | I | R |
| Ampicillin | 39 | N/A* | 61 | 34 | 5 | 61 | 40 | 3 | 57 |
| Amoxicillin-clavulanate | 79 | N/A* | 21 | 71 | 18 | 11 | 62 | 36 | 2 |
| Cephalexin | 91 | N/A* | 9 | 91 | 0 | 9 | 92 | 4 | 4 |
| Ciprofloxacin | 79 | 4 | 17 | 85 | 0 | 15 | 77 | 9 | 14 |
| Nitrofurantoin | 100 | N/A* | 0 | 97 | 1 | 2 | 91 | 9 | 0 |
| Trimethoprim | 70 | 0 | 30 | 70 | 0 | 30 | 68 | 0 | 32 |

*EUCAST guidelines for ampicillin, amoxicillin-clavulanate, nitrofurantoin and cephalexin do not include intermediate zones.

the results obtained from the Stokes technique and the results derived from the CLSI and EUCAST methods, and they were tested for each of the six antimicrobial agents used in the study (180 discs). For 40.6% of these inoculum standardised repeats ($n$ = 73), the same zone diameter was recorded. For 51.7% of the inoculum standardised repeats performed ($n$ = 93), a different zone diameter was recorded. However, the interpretive result in these cases (Susceptible, Intermediate or Resistant) remained the same as that obtained in the initial test result. The standardisation of the inoculum affected the interpretative result of the remaining 14 duplicates; interpretation for 5% ($n$ = 9) of these changed from Intermediate to Sensitive when the standardised inoculum rather the direct inoculation with urine sample was used, 1.7% ($n$ = 3) demonstrated a change in interpretation from Resistant to Sensitive, 0.6% ($n$ = 1) demonstrated a change in interpretation from Intermediate to Resistant, while the remaining 0.6% ($n$ = 1) demonstrated a change from Sensitive to Intermediate susceptibility. The 14 antimicrobial results which changed using standardised repeats were as follows: amoxicillin-clavulanate $n$ = 5, ciprofloxacin $n$ = 4, nitrofurantoin $n$ = 2, trimethoprim $n$ = 2 and ampicillin $n$ = 1. Susceptibility interpretations for cephalexin were unaffected by inoculum standardisation.

The results of a precision blind trial using duplicate susceptibility testing and representing the six antimicrobial agents used in the study showed a 94.8% ($n$ = 328) agreement of breakpoint zone diameters between the 346 duplicated disc tests being observed with indistinguishable zone sizes. A 1 mm deviation between the duplicate zone size measurements was observed in 4% ($n$ = 14) of the isolates, and a 2 mm deviation between the measurements of the duplicates was observed in the remaining 1.2% ($n$ = 4) of the isolates.

There were varying levels of agreement between the three AST methods (Table 2). The highest level of agreement across the three interpretive parameters can be seen with trimethoprim (98%). Trimethoprim and cephalexin were recorded as having 100% agreement between EUCAST and CLSI, with the only discrepancies arising from the Stokes method interpretation. Conversely, ampicillin had a slightly greater level of agreement between CLSI and Stokes AST methods (96%) than between CLSI and EUCAST (95%). The poorest agreement across the three interpretive guidelines was observed in relation to amoxicillin-clavulanate (60% agreement only). Complete disagreement between the three AST methods was observed for two isolates for ciprofloxacin and for two isolates for nitrofurantoin.

The effect of changes in interpretive breakpoint guidelines in EUCAST and CLSI on the level of agreement between methodologies was determined. Any investigation of the agreement between the three AST methods studied here requires consideration of revised breakpoint guidelines over time (CLSI and EUCAST). The impact of changes in breakpoints from 2011–2017 can be observed among three of the antimicrobial agents tested (Table 3). As an example, taking cephalexin/cephradine the proportion that show complete agreement across the three methods is much higher (95%) using 2015 and 2017 interpretative guidelines, than that when using the older, 2011 and 2013 guidelines (80%).

## Discussion

Although the aetiology of UTI remains largely consistent, epidemiological knowledge of local susceptibility trends is an integral component of appropriate empirical treatment of UTI [18]. This is important, as failing to administer antibiotics or utilising antibiotics to which the organism is resistant results in 50–60% longer duration of symptoms [3]. UTI-associated *E. coli* was selected for this study on the basis that UPEC causes around 90% of community-acquired UTIs and up to 50% of nosocomial UTIs [19].

A total of 45% of the isolates generated an identical AST result for the six antimicrobials tested, irrespective of the AST method used. However, in the case of a further 38% of the isolates, five of the six antimicrobials gave an identical result (Table 1). It should be noted that within the EUCAST guidelines, the intermediate category is not employed for ampicillin, amoxicillin-clavulanate, nitrofurantoin and cephalexin. The greatest differences in resistance rates occurred for amoxicillin-clavulanate, showing resistances of 21% (EUCAST), 11% (CLSI) and 2% (Stokes). The AST interpretation of CLSI and Stokes rates for amoxicillin-clavulanate were complicated by the large proportion of results that were intermediate, rather than sensitive or resistant. In the case where resistance was extended to include intermediate or reduced susceptibility, these figures became 21% (EUCAST), 29% (CLSI) and 38% (Stokes). This means, the three methods could potentially lead to the recommendation of different treatment regimens for the same *E. coli* isolate.

Table 2 provides a representation of the levels of agreement for the six antimicrobials tested in the study

**Table 2.** Representation of the levels of susceptibility agreement among 100 uropathogenic E. coli isolates for six oral antimicrobial agents.

| Antimicrobial agent | EUCAST/CLSI/Stokes agreement | EUCAST/CLSI agreement only | CLSI agreement only | EUCAST/Stokes agreement only | No agreement |
|---|---|---|---|---|---|
| Ampicillin | 91 | 4 | 5 | 0 | 0 |
| Amoxicillin-clavulanate | 60 | 22 | 14 | 4 | 0 |
| Cephalexin | 89 | 5 | 1 | 3 | 2 |
| Ciprofloxacin | 95 | 5 | 0 | 0 | 0 |
| Nitrofurantoin | 91 | 6 | 1 | 0 | 2 |
| Trimethoprim | 98 | 2 | 0 | 0 | 0 |

**Table 3.** Effect of the changes in interpretive guidelines on the level of agreement observed for CLSI, EUCAST and Stokes Antimicrobial Susceptibility Test methods.

| | A | B | C | D | E |
|---|---|---|---|---|---|
| *Amoxicillin-clavulanate* | | | | | |
| 2017 | 60 | 22 | 14 | 4 | 0 |
| 2015 | 60 | 22 | 14 | 4 | 0 |
| 2013 | 60 | 22 | 14 | 1 | 3 |
| 2011 | 60 | 22 | 14 | 1 | 3 |
| *Ciprofloxacin* | | | | | |
| 2017 | 89 | 5 | 1 | 3 | 2 |
| 2015 | 90 | 10 | 0 | 0 | 0 |
| 2013 | 90 | 10 | 0 | 0 | 0 |
| 2011 | 90 | 10 | 0 | 0 | 0 |
| *Cephalexin-cephradine* | | | | | |
| 2017 | 95 | 5 | 0 | 0 | 0 |
| 2015 | 95 | 5 | 0 | 0 | 0 |
| 2013 | 80 | 5 | 0 | 15 | 0 |
| 2011 | 80 | 4 | 0 | 16 | 0 |

Notes: Data are percentages. A = Agreement across the three guidelines, B = CLSI and EUCAST agree, C = CLSI and Stokes agree, D = EUCAST and Stokes agree, E = no agreement across the three guidelines. Interpretive guidelines were changed for these antimicrobial options between 2011 and 2017. The interpretive guidelines for ampicillin, nitrofurantoin and trimethoprim remain unchanged.

when using the interpretive guidelines for the three methods. The Stokes result differed from the other two methods for 48 antimicrobial agent tests (8%). However, for a further 28 antimicrobial agent tests (4.7%), the Stokes method accorded with either EUCAST or CLSI. It may be useful to compare the results of this study for current EUCAST (2017) guidelines with those of CLSI (2017) for UPEC as these are the two most widely employed disc diffusion methods and guidelines used in clinical laboratories currently. Extrapolating from Table 2, the level of accordance when following the 2017 guidelines for EUCAST and CLSI was as follows: ampicillin: 95%; amoxicillin-clavulanate: 82%; ciprofloxacin: 94%; cephradine/cephalexin: 100%; nitrofurantoin: 97% trimethoprim: 100%. The fact that standardising the inoculum did not alter the interpretive result in 92.3% of the duplicates indicated that inoculum density may not have as significant role as might have been anticipated in the discrepancies observed when compared to EUCAST and CLSI results. It may be worth mentioning here, however, that in accordance with the CLSI (2008) [20] these susceptibility classifications would be deemed as a minor error in 6% of cases, and as a major error in 1.7% of cases, the latter being where a change from resistant to sensitive or sensitive to resistant had occurred.

EUCAST and CLSI make annual changes to the breakpoint zone diameters in their respective interpretive guidelines through the use of pharmacokinetics studies, examination of resistance mechanisms, MIC distributions, zone diameter distributions, pharmacodynamics and epidemiological cut-off values [11,17]. The resulting adjustments to breakpoint guidelines might be expected to make it more challenging to accurately compare the AST data from previous years with some more recent results. In the current study, as shown in Table 3, which compares interpretations of S/I/R over four periods between 2011 and 2017 inclusive, there are three agents which have had changes in their EUCAST or CLSI interpretive guidelines since 2011; amoxicillin-clavulanate, ciprofloxacin and cephalexin. The susceptibility results of these agents have been interpreted using four sets of annual guidelines to evaluate the effect of the adjustment in breakpoint zone diameters on AST results (Table 3). For amoxicillin-clavulanate, the changes in EUCAST interpretive breakpoint guidelines mean that if the 100 isolates were interpreted using the 2011 or 2013 guidelines, that three of the isolates would have no agreement between the three methods, in contrast to complete agreement when interpreted with the 2015 or 2017 guidelines. Conversely, the narrowing of the CLSI breakpoint zone diameters in 2014 and the raising of the EUCAST breakpoints for cephalexin in 2013 (Table 3) has caused an immediately noticeable effect, whereby using the 2015 and 2017 interpretive guidelines the proportion of the 100 isolates that show complete agreement across the three methods is much higher (95%), than the number observed when using the older, 2011 and 2013 methods (80%). The changes also reduce the number of isolates demonstrating agreement between EUCAST and Stokes only, from 16 to 0. As the interpretive guidelines for trimethoprim, nitrofurantoin and ampicillin have remained unchanged since 2011 for EUCAST and CLSI methods there are no breakpoint-induced changes in reported resistance levels.

In conclusion, our findings indicate that a comparison of susceptibility rates can only be considered after ascertaining the degree of discrepancy generated through using different AST methods, and different interpretive guidelines. This is very concerning considering that amoxicillin-clavulanate is most affected in this regard and is the most commonly prescribed antimicrobial administered for

this condition in Ireland (used in 33.1% of cases) [21]. These data indicate unwelcome complications for best antimicrobial stewardship and prescribing practices. This work represents an advance in biomedical science because it provides novel information on the comparability of susceptibility test methods and interpretive guidelines for UPEC.

## Summary table

*What is known about the subject*

- There is a choice of susceptibility test methods available for clinical laboratories
- Susceptibility and resistance are reported according to pre-determined interpretive guidelines in each case
- Interpretive guidelines may change for a single method over time

*What this study adds*

- Inter-method variability can cause different reported rates of susceptibility when applied to the same population of uropathogenic *E. coli* test strains
- Variations in interpretive guidelines have given rise to different reported susceptibility rates when applied to the same test strain population
- The variance in reported susceptibility rates among uropathogenic *E. coli* strains is most marked for amoxycillin-clavulanate and ciprofloxacin among oral antimicrobial agents commonly used to treat urinary tract infection

## Acknowledgements

The authors also wish to acknowledge their colleagues in the Clinical Microbiology Department of Cork University Hospital for their support of this work, and particularly that of Mr. Eddie McCullagh, Senior Medical Scientist.

## Disclosure statement

This work was conducted on anonymised patient samples, after ethical approval had been granted. There was no conflict of interest.

## Funding

Funding was provided jointly by the Biological Sciences Department of Cork Institute of Technology and the Microbiology Department at Cork University Hospital.

## ORCID

*C O'Halloran* http://orcid.org/0000-0002-8869-9826

## References

[1] Stamm WE. Scientific and clinical challenges in the management of urinary tract infections. Am J Med. 2002;113(Suppl 1A):1S–4S.

[2] Little P, Merriman R, Turner S, et al. Presentation, pattern, and natural course of severe symptoms, and role of antibiotics and antibiotic resistance among patients presenting with suspected uncomplicated urinary tract infection in primary care: observational study. BMJ. 2010;340:b5633.

[3] Guay DR. Contemporary management of uncomplicated urinary tract infections. Drugs. 2008;68(9):1169–1205.

[4] Llor C, Bjerrum L. Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem. Ther Adv Drug Saf. 2014;6:229–241.

[5] Biemer JJ. Antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method. Ann Clin Lab Sci. 1973;3(2):135–140.

[6] Guidoni EBM, Berezin ENS, et al. Antibiotic resistance patterns of pediatric community-acquired urinary infections. Braz J Infect Dis. 2008;12(4):321–323.

[7] EUCAST. (2012). Direct antimicrobial susceptibility testing [Internet]. Available from: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/General_documents/Direct_testing_guidance_note_Feb2012.pdf

[8] Gould IM. Towards a common susceptibility testing method? J Antimicrob Chemother. 2000;45(6):757–762.

[9] Dudley MN., Ambrose PG., Bhavnani SM., et al. Background and rationale for revised clinical and laboratory standards institute interpretive criteria (breakpoints) for *Enterobacteriaceae* and *Pseudomonas aeruginosa*: I Cephalosporins and Aztreonam. Clin Infect Dis. 2013;56(9):1301–1309.

[10] Wolfensberger A., Sax H., Weber R., et al. Change of antibiotic susceptibility testing guidelines from CLSI to EUCAST: Influence on cumulative hospital antibiograms. PLoS ONE. 2013;8(11):e79130.

[11] EUCAST. (2016b). Setting breakpoints for new antimicrobial agents. Available from: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/EUCAST_SOPs/EUCAST_SOP_1.2_Setting_breakpoints_new_agents_20161121.pdf

[12] Matuschek E., Brown DFJ., Kahlmeter G.. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. Clin Microbiol Infect. 2014;20(4):O255–O266.

[13] Doern GV. Antimicrobial susceptibility testing. J Clin Microbiol. 2011;49(9):S4.

[14] Gosden PE., Andrews JM., Bowker KE., et al. Comparison of the modified stokes' method of susceptibility testing with results obtained using MIC methods and British Society of Antimicrobial Chemotherapy breakpoints. J Antimicrob Chemother. 1998;42(2):161–169.

[15] CLSI. (2012). M02-A11 - Performance standards for antimicrobial disk susceptibility tests; Approved Standard – Eleventh Edition. Available from: https://www.bing.com/search?q=performance+standards+for+antimicrobial+disk+susceptibility+tests%3B+approved+standard+%E2%80%93+eleventh+edition&form=EDGTCT&qs=PF&cvid=3a31ee62a0ce4db6b60242275a1163a5&cc=GB&setlang=en-US

[16] EUCAST. (2017). EUCAST disk diffusion method for antimicrobial susceptibility testing Version 6.0. Available from: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/Version_5/Slide_show_v_6.0_EUCAST_Disk_Test.pdf

[17] CLSI. (2017). M100 - Performance standards for antimicrobial susceptibility testing - 27th edition. Available from: http://em100.edaptivedocs.info/GetDoc.aspx?doc=CLSI%20M100%20S27:2017&scope=use

[18] Ronald A. The etiology of urinary tract infection: traditional and emerging pathogens. Disease-a-Month. 2003;49(2):71–82.

[19] Kucheria R., Dasgupta P., Sacks H., Khan M., Sheerin N.. Urinary tract infections: new insights into a common problem. Postgrad M J. 2005;81(952):83–86.

[20] CLSI. (2008). Development of *in vitro* susceptibility testing criteria and quality control parameters. 3d edition. M23-A3. Wayne (PA): Clinical and Laboratory Standards Institute.

[21] Vellinga A., Cormican M., Hanahoe B., et al. Antimicrobial management and appropriateness of treatment of urinary tract infection in general practice in Ireland. BMC Fam Pract. 2011;12(1):143.