

Antimicrobial resistance of bacteria isolated from urinary tract infections in women

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Condensation:
This paper describes the
antimicrobial resistance
of uropathogens isolated
during a two-years period;
the results supported
an update of treatment
recommendation for urinary
tract infections.

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Abstract

Variability in antibiotic resistance of microorganisms commonly involved in urinary tract infections requires regularly updates to determine appropriate treatment options. **Methods.** we analyzed resistance profiles for 2043 non-duplicate bacterial strains isolated from urinary tract infections in women hospitalized in INBI “Matei Balș” between July 2008 - June 2010. The level of bacterial resistance and risk of fetal toxicity defined a limited number of therapeutic options for urinary tract infections in pregnancy. **Results.** The most strains were gram negative bacilli, 88,50%, *Escherichia coli* accounting for 81,80% of them. 11,50% strains were gram positive cocci, 57,44% being *Enterococcus faecalis*. Carbapenems and nitrofurantoin remain highly active (except for gram-negative nonfermentative bacteria); piperacillin-tazobactam and 3rd-4th generation cephalosporins had relatively good activity; aminopenicillins-betalactamases inhibitors association activity was low. The preliminary results show the novelty in antimicrobial treatment recommendations in pregnancy. **Conclusions.** The antimicrobial resistance data support antimicrobial treatment recommendations in pregnancy for asymptomatic bacteriuria, cystitis (nitrofurantoin, fosfomycin) and for pyelonephritis - 3rd generation cephalosporins (carbapenems for nosocomial infections).

Keywords: urinary tract infections, antimicrobial resistance, treatment guidelines

Urinary tract infections represent one of the most common infectious pathologies in women, 20-40% of them presenting at least one episode during lifetime⁽¹⁾. Knowing the susceptibility profiles of the frequently involved microorganisms in their etiology is useful in order to establish treatment options based on local resistance data; for pregnant women these data are especially important as some of the antibiotics allowed in pregnancy and classically recommended for the treatment of urinary infections⁽²⁾ could be inactive against bacterial strains currently in circulation (i.e. ampicillin for *Escherichia coli*⁽³⁾). If such a situation is noticed, the therapeutic recommendations for asymptomatic bacteriuria and urinary tract infections in pregnant women must be updated according to the present antimicrobial resistance patterns.

Objectives

To analyse the antimicrobial susceptibility patterns of the bacterial strains isolated from urinary tract infections in women, in order to establish the appropriate therapeutic options.

Methods

We analysed the antimicrobial susceptibility tests for uropathogens isolated in women hospitalized in INBI „Matei Balș” between 1st July 2008 - 30 June 2010. The data were used as they were communicated to clinicians, without making any additional considerations about resistance profiles (for example, without an analysis of the antimicrobial susceptibility profile in order to suppose the

presence of ESBL positive strains even this situation was not indicated by the microbiology laboratory).

Inclusion criteria: We considered useful to review all obtained resistance profiles for bacteria isolated from women with urinary infection, regardless the presence of pregnancy, whereas no etiological features in pregnant women, and the analysis of a larger volume of data allowed us to obtain results with a higher statistic significance.

Exclusion criteria: The duplicated strains, defined as the same strain isolated from urine within the first 4 weeks from the first isolation, were excluded from the analysis.

The statistical analysis of the data was performed with EPIINFO 3.4.3.

Results

1. The distribution of the isolated strains

2043 strains were isolated. Most of them were gram negative bacilli: 1808 strains (88.50%), from which 1774 strains (98.12%) were *Enterobacteriaceae* and 34 strains (1.88%) were nonfermentative gram negative bacteria. For *Enterobacteriaceae* we used a classification used by other surveillance systems (4): *E. coli*, *Klebsiella spp.*, *Proteus-Morganella-Providencia spp.*, *Enterobacter-Citrobacter spp.*, other *Enterobacteriaceae*. The most frequently isolated was *E. coli* (1479 strains, 72.39%). *Klebsiella spp* (195 strains, 9.54%), *Enterobacter-Citrobacter spp.* (40 strains, 1.96%), *Proteus-Morganella-Providencia-spp.* (56 strains, 2.74%), other *Enterobacteriaceae* (4 strains, 0.2%) were isolated in lower proportion.

Gram-negative nonfermentative isolated bacteria were *Pseudomonas spp.* (28 strains, 1.37%) and *Acinetobacter baumannii* (6 strains, 0.29%).

We identified 235 strains of gram-positive cocci (11.50% of positive urine cultures). They were: *Enterococcus spp.* (195 strains, 9.54% of total isolates), *Staphylococcus spp.* (14 strains, 0.69% of total isolates) and group *B. streptococci* (26 strains, 1.27% of total isolates) (Figure 1).

2. *E. coli* antimicrobial susceptibility

We identified 1479 strains of *Escherichia coli* for which we analyzed the susceptibility data for the antimicrobials that we considered to be important from both epidemiological and clinical point of view, and also have no proven fetal toxicity: ampicillin, associations of aminopenicillin-betalactamase inhibitor, piperacillin-tazobactam, 2nd and 3rd generation cephalosporins, carbapenems, and nitrofurantoin. We evaluated the same antimicrobials for the other *Enterobacteriaceae* identified strains.

The antimicrobials susceptibility data analysis for *E. coli* strains indicated (Figure 2):

- aminopenicillins can no longer be a first-line therapy (with a susceptibility of only 40.75%), nevertheless, they can be used to narrow the activity spectrum in the cases with proven susceptibility of *E. Coli* to these antimicrobials;

- the associations with beta-lactamase inhibitors partially restores the effectiveness of penicillins. In our study the susceptibility rate for aminopenicillin-betalactamase inhibitor association was 70.79%, and such a low level of activity compromises their usefulness as a first line treatment option for urinary tract infections. The susceptibility rate for piperacillin-tazobactam was 87.52% and this good level of activity (close to 3rd generation cephalosporins activity), makes this association one of the preferred options for the first line treatment;

- the susceptibility rate for ceftriaxone was 89.96%. For the most of the resistant strains, this was due to ESBL production;

- the carbapenems activity remains excellent, with a susceptibility rate of 99.72%; however, because the resistance of *E. coli* to this antimicrobials class is rare, the presence of 4 isolates with diminished susceptibility, or resistant to carbapenems could be explained by testing errors. In order to evaluate the emergence of carbapenem-resistant strains of *E. coli*, major problem for public health and especially for nosocomial infections control, is necessary to verify the presence of such strains with MIC determination;

- the high level of susceptibility to nitrofurantoin - 96%, makes this antibiotic one of the best choices for the first line treatment for urinary tract infections.

3. *Klebsiella spp.* antimicrobial susceptibility

We identified 195 strains belonging to *Klebsiella spp.*; among them, 162 strains were *Klebsiella pneumoniae*. The analysis of the susceptibility rates among these isolates highlighted the following changes of in vitro activity (Figure 3):

- the extremely low level of activity for aminopenicillins (4.21%) excludes them from the treatment options for urinary tract infections due to *Klebsiella spp.*;

- 3rd generation cephalosporins had a lower activity in this case (72.45%) than that against *E. coli*;

- as with *E. coli*, the association with beta-lactamase inhibitors partially restored penicillin efficiency; 57.51% of strains were susceptible to amoxicillin-clavulanate, yet too few to be recommended for the treatment of urinary tract infections caused by *Klebsiella spp.* Piperacillin-tazobactam, at a rate of 62.43% *in vitro* activity, had a statistically insignificant superiority to that of betalactamase aminopeniciline-inhibitors associations;

- although carbapenems have excellent activity against *Klebsiella spp.* (99.47%), reporting a resistant strain should

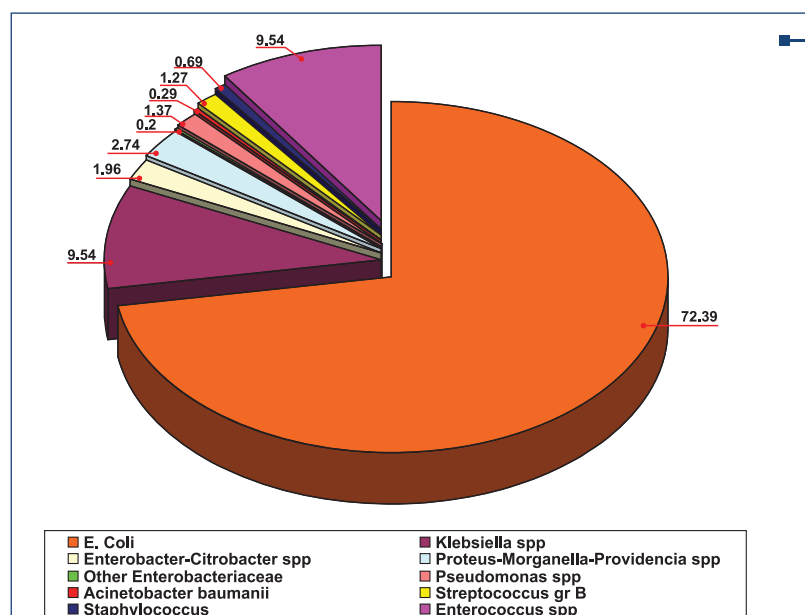


Figure 1. The proportion of uropathogenic strains isolated in women: *E. coli* (72.39%), *Klebsiella spp.* (9.54%), *Enterobacter-Citrobacter spp.* (1.96%), *Proteus-Morganella-Providencia spp.* (2.74%), *other Enterobacteriaceae* (0.2%), *Pseudomonas spp.* (1.37%), *Acinetobacter baumannii* (0.29%), *Enterococcus spp.* (9.54%), *Staphylococcus spp.* (0.69%), *group B. streptococci* (1.27%)

be confirmed by MIC determinations before being reported, in order to avoid extensive measures for infections control that would be mandatory in case of the identification of carbapenemases producing *Enterobacteriaceae* strains;

- nitrofurantoin activity was much lower compared to that against *E. coli*: 36.97%;

4. Enterobacter-Citrobacter group antimicrobial susceptibility

We identified 40 strains belonging to *Enterobacter-Citrobacter* group, 34 strains being *Enterobacter spp.*

The analysis of these germs' antimicrobial susceptibility revealed (Figura 4):

- a low activity, identical for 3rd generation cephalosporins and piperacillin-tazobactam (67.56%);

- for carbapenems, the susceptibility rate was 92.3%, because of the 3 resistant isolates. Althow the activity rate

is very good, the number of resistant strains is worrying and requires confirmation through CMI determinations before being reported; the confirmation raises the major risk of missing back-up therapeutic solutions for the *Enterobacteriaceae* infections;

- the activity of aminopenicillins is minimal: 5.26%. No improvement is obtained by adding a betalactamase inhibitor. Susceptibility to amoxicillin-clavulanate was 15.78% (p = 0.13);

- nitrofurantoin showed a low activity - 38.89%, which makes it not recommendable for urinary tract infections with this particular etiology.

5. Proteus-Morganella-Providencia group antimicrobial susceptibility

56 *Proteus-Morganella-Providencia spp.* strains were identified, mostly belonging to *Proteus spp.* (51 strains). Only 4 *Morganella spp.* strains were identified.

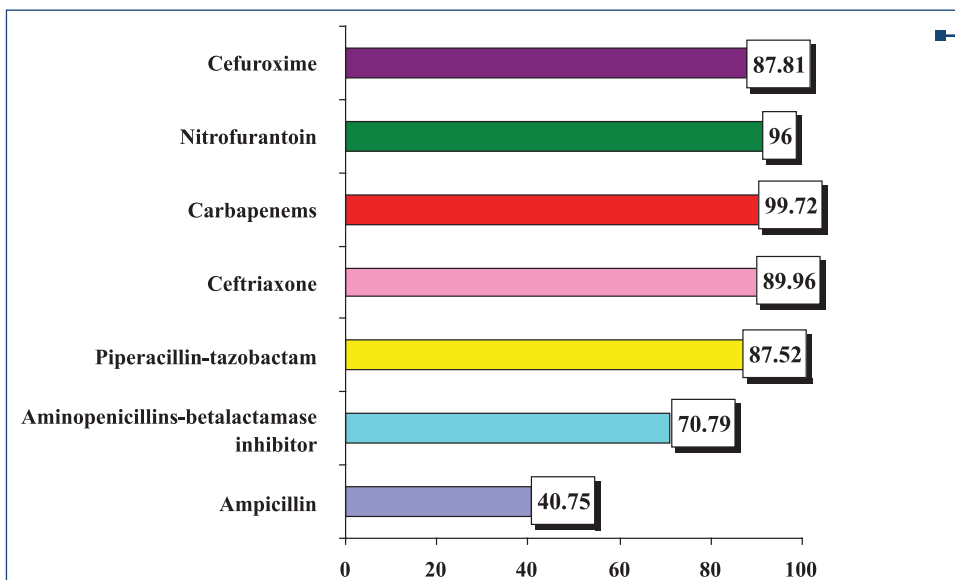


Figure 2. Antimicrobial susceptibility of *E. coli* strains: aminopenicillins (40.75%), aminopenicillins- beta-lactamase inhibitors associations (70.79%), piperacillin-tazobactam (87.52%), ceftriaxone (89.96%), cefuroxime (87.81%), carbapenems (99.72%), nitrofurantoin (96%)

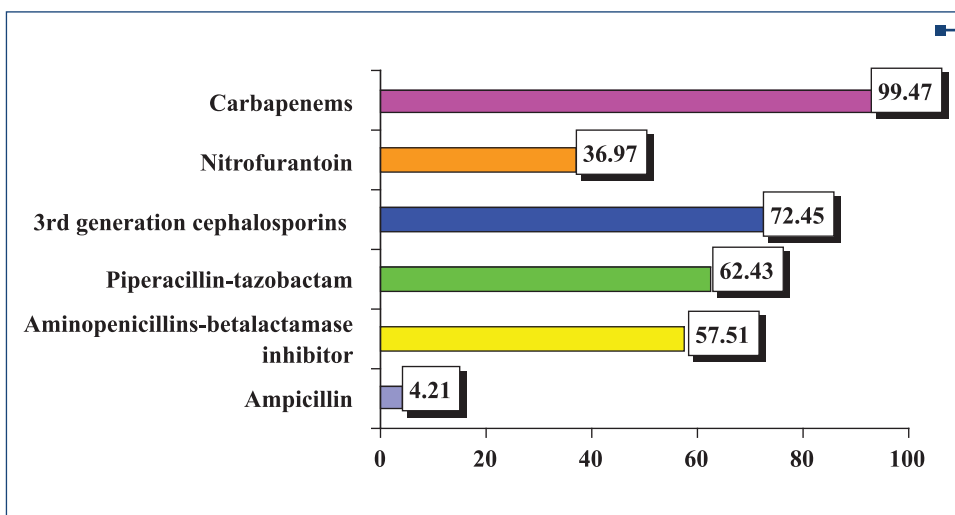


Figure 3. Antimicrobial susceptibility of *Klebsiella spp.* strains: aminopenicillins (4.21%), aminopenicillins-beta-lactamase inhibitors associations (57.51%), piperacillin-tazobactam (62.43%), 3rd generation cephalosporins (72.45%), carbapenems (99.47%), nitrofurantoin (36.97%)

The analysis of *Proteus-Morganella spp* resistance to antibiotics revealed the following aspects (Figure 5):

- 3rd generation cephalosporins recorded an activity of 69.77%;

- although carbapenems prove a very good activity, signaling a resistant strain requires the same precautions as for the previous described situations;

- with 35.18% susceptibility level, aminopenicillins can no longer represent first line therapy for the urinary tract infections caused by these bacteria. Not even the association with a betalactamase inhibitor, which partially restores their efficiency (49.05%), can not represent a suitable therapeutic alternative in these cases.

A very good activity was shown by piperacillin-tazobactam: 85.18%. As one of the alternatives to carbapenems in the first line therapy for this particular germ group, it

represents „the second best option”, as several medical systems have agreed.

6. *Pseudomonas spp.* antimicrobial susceptibility

28 strains of *Pseudomonas spp.* were identified. Their susceptibility was assessed for antibiotics considered to be clinically and epidemiologically relevant: ceftazidim, piperacillin-tazobactam, carbapenems, aztreonam, colistin.

The analysis of *Pseudomonas spp.* antimicrobial susceptibility revealed extremely low activity levels for most evaluated antibiotics (Figure 6):

- ceftazidim’s activity was 26.92%, very close to that of piperacillin-tazobactam: 29.63%;

- in the case of carbapenems, a low susceptibility rate was recorded: 35.71%. Although the meropenem is known to be more active than imipenem against *Pseudomonas spp.*, in this case, it has shown a much lower activity: 16.66%.

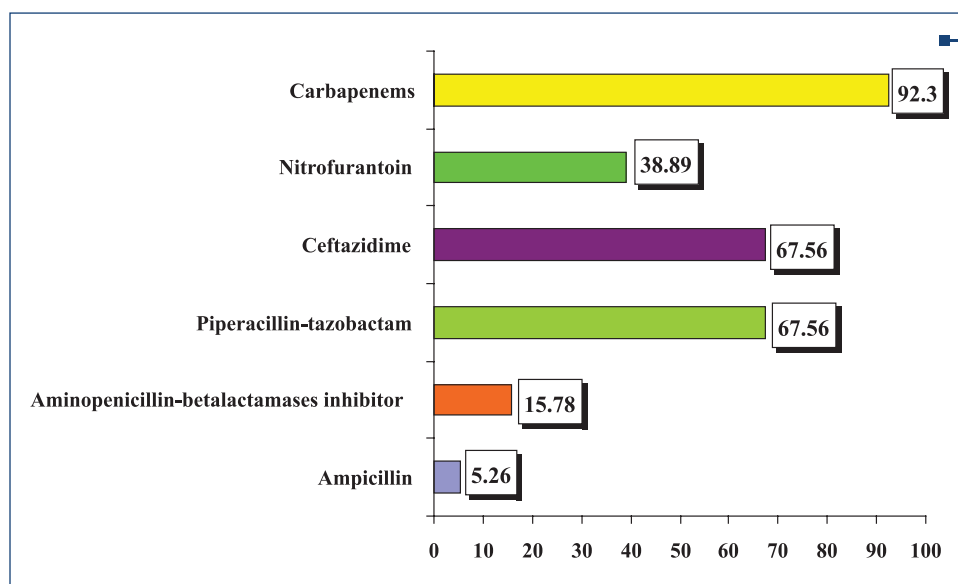


Figure 4. Antimicrobial susceptibility of *Enterobacter-Citrobacter spp.* strains: aminopenicillins (5.26%), aminopenicillins-beta-lactamase inhibitors associations (15.78%), piperacillin-tazobactam (67.56%), nitrofurantoin (38.89%), 3rd generation cephalosporins (67.56%), carbapenems (92.3%)

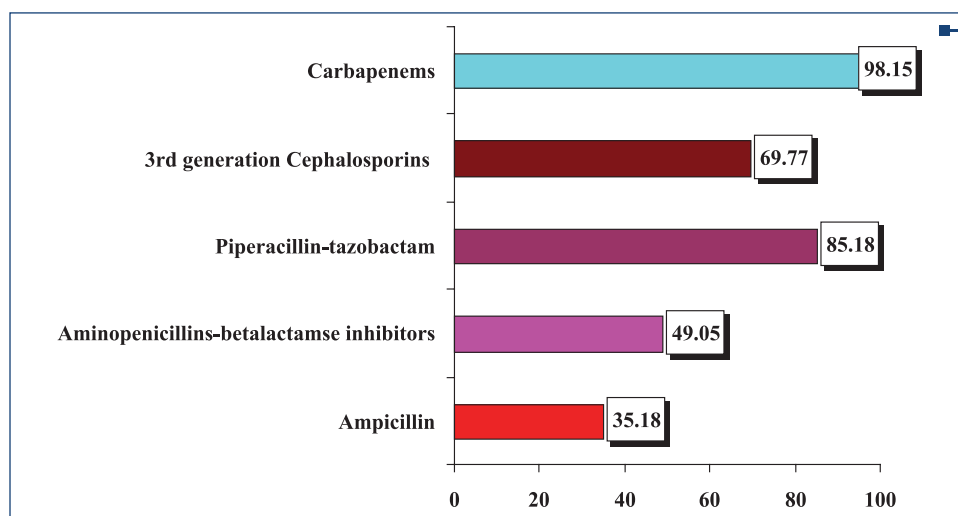


Figure 5. Antimicrobial susceptibility of *Proteus-Providencia-Morganella spp.* strains: 3rd generation cephalosporins (69.77%), carbapenems (98.15%), aminopenicillins (35.18%), aminopenicillins-beta-lactamase inhibitors associations (49.05%), piperacillin-tazobactam (85.18%)

The probable cause is the small number of strains tested for meropenem: only one of the six tested isolates was susceptible. By objectifying the low susceptibility rates for these antibiotics, a phenomenon long known in other regions is confirmed: the loss of carbapenem supremacy, secondary to the extension of their use as first intention treatment;

- only 3 strains were tested for aztreonam, and only 1 proved to be susceptible;

- colistin, considered a rescue therapy against non-fermentative gram negative bacilli, showed an efficiency rate of 84.61%.

Fortunately, very few pregnant women are at risk of having such an infection because urinary tract infections caused by *Pseudomonas* are usually hospital-acquired, especially after urological interventions, a situation rarely seen in young adult women.

7. *Acinetobacter baumannii* antimicrobial susceptibility

Only 6 *Acinetobacter baumannii* strains were isolated from urine cultures. Their susceptibility was assessed for ampicillin-sulbactam and carbapenems. The low rate of identified strains, and even lower rate of tested strains for some antibiotics rendered the analysis difficult. None of the strains proved susceptible to ampicillin-sulbactam,

and one strain was susceptible to each one of the other tested antibiotics (Table 1).

8. *Enterococcus spp.* antimicrobial susceptibility

195 strains of *Enterococcus spp.* were identified: 135 strains of *Enterococcus faecalis* and 51 strains of *Enterococcus faecium*. In 9 cases only *Enterococcus spp.* was specified. The susceptibility was investigated for a series of antibiotics considered to be clinically and epidemiologically relevant: ampicillin, nitrofurantoin, glycopeptides and linezolid.

a. *Enterococcus faecalis*

In the case of *Enterococcus faecalis*, the antibiotic resistance profile analysis revealed (Figure 7):

- for ampicillin, the susceptibility level was 92.42%. This value indicates the possibility of its use as a first line treatment against urinary tract infections with this etiology;

- the susceptibility to linezolid was 99.21%, with only one strain described as intermediary susceptible; such a situation imposes a thorough CMI verification, before reporting the results obtained through (semi)automatic methods;

- the susceptibility to vancomycin was 100%;

- the absence of the vancomycin-resistant strains and the extremely low number of strains resistant to linezolid

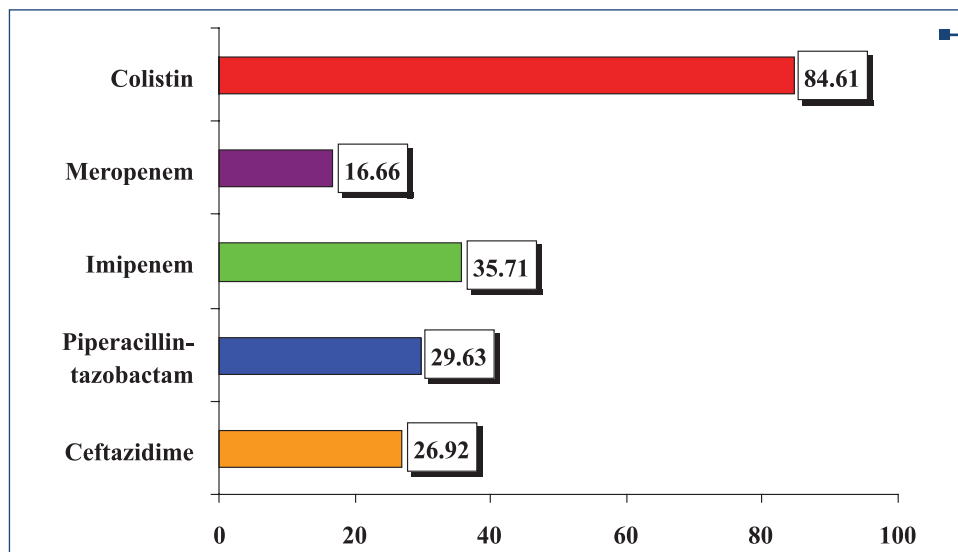


Figure 6. Antimicrobial susceptibility of *Pseudomonas spp.* strains: ceftazidime (26.92%), piperacillin-tazobactam (29.63%), imipenem (35.71%), meropenem (16.66%), colistin (84.61%)

Table 1

Antimicrobial susceptibility of *Acinetobacter baumannii* strains: ampicillin-sulbactam (no susceptible strain of 3 tested strains), imipenem (1 susceptible strain of 6 tested strains), meropenem (1 susceptible strain of 3 tested strains)

Tested antimicrobial	Number of tested strains/number of isolated strain	Number of resistant strains/number of tested strains
Ampicillin-sulbactam	3/6	0/3
Imipenem	6/6	1/6
Meropenem	3/6	1/3

indicates a favourable situation for the treatment of the infections due to ampicillin resistant isolates;

- the good level of efficiency for nitrofurantoin (87.93%) recommends it as an alternative for urinary tract infections in which *Enterococcus faecalis* may be involved.

b. *Enterococcus faecium*

The susceptibility analysis of *Enterococcus faecium* revealed some different aspects compared to *Enterococcus faecalis* (Figure 8):

- for ampicillin, unlike *E. faecalis*, the resistance is almost constant; the susceptibility level is very low, 7.84%;

- the activity of nitrofurantoin is lower than in the case of *Enterococcus faecalis*: 61.9%;

- linezolid recorded a very high susceptibility rate: 95.83%; the 2 strains described as intermediary susceptible require a thorough CMI verification;

- no vancomycin resistant strains were identified; the situation is similar to that in other European countries; the enterococci's resistance to glycopeptides is more frequently described in the United States⁽⁵⁾ and only a few regions of Europe⁽³⁾ (i.e. Greece).

The high activity level of vancomycin and linezolid, „back-up” antibiotics for the treatment of enterococcal infections, is gratifying and a control of their use could help to maintain this situation.

Unfortunately, both linezolid and glycopeptides are considered category “C” of fetal risk and therefore their use is still reserved for some cases where the benefit outweighs the risk (for instance in pyelonephritis or complicated urinary tract infections).

9. The antimicrobial susceptibility of group B *Streptococcus* strains obtained from urine cultures

The *Streptococcus* group B is can frequently colonize asymptotically the urethra of both male and female patients.

Simptomatic urinary tract infections determined by these germs are usually associated with pregnancy, or found in patients presenting risk factors. In this case, antibiotic treatment is taken into consideration. For this reason, this study includes the susceptibility analysis for this bacteria. 26 group B *Streptococcus* strains were identified, isolated from urine cultures, in women. For these strains, the susceptibility profiles were analysed

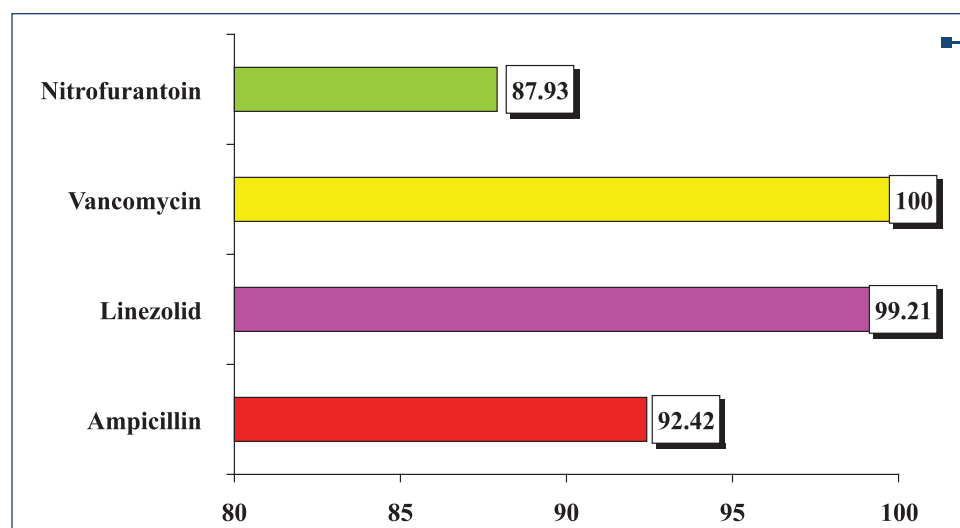


Figure 7. Antimicrobial susceptibility of *Enterococcus faecalis* strains: ampicillin (92.42%), linezolid (99.21%), vancomycin (100%), nitrofurantoin (87.93%)

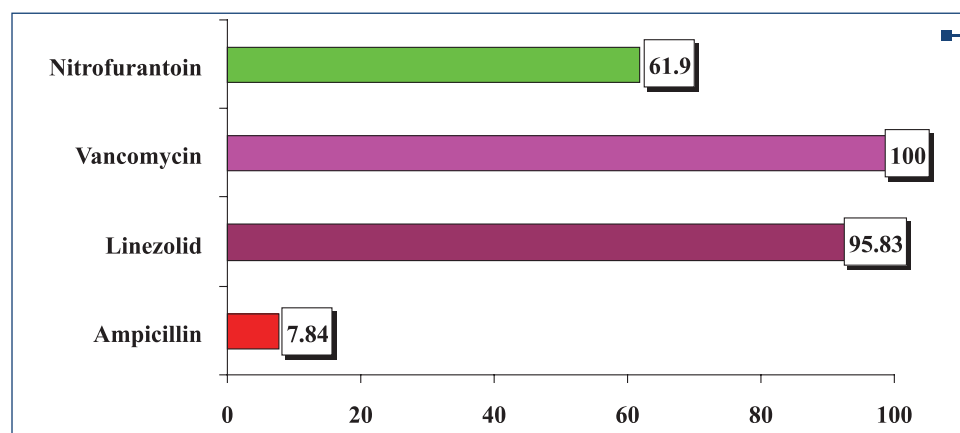


Figure 8. Antimicrobial susceptibility of *Enterococcus faecium* strains: ampicillin (7.84%), nitrofurantoin (61.9%), linezolid (95.83%); vancomycin (100%)

for penicillin, macrolides, clindamycin, glycopeptides, linezolid, nitrofurantoin.

Figure 9 presents the susceptibility profile of group B *Streptococcus* strains to the analysed antibiotics:

- no strains proved resistant to clindamycin, rifampin, glycopeptides, or nitrofurantoin. This offers sufficient therapeutic alternatives for this type of urinary tract infections;
- the susceptibility rate to penicillin was 95%; only 2 strains proved resistant;
- a low level of resistance was recorded in the case of macrolides (78.57%);
- one strain was identified as intermediary susceptible to linezolid; in the absence of additional tests for MIC verification, this could represent a testing error.

10. The antimicrobial susceptibility of *Staphylococcus spp.* strains obtained from urine cultures

Only 14 *Staphylococcus spp.* strains were identified: 6 strains were *Staphylococcus aureus* and 8 strains were coagulase-negative *staphylococci*.

In the case of these isolates, the susceptibility profile was analysed for penicillin, oxacillin, macrolides, clindamycin, glycopeptides, linezolid.

The susceptibility analysis for these antibiotics revealed (Figure 10):

- 100% susceptibility rate for three of the evaluated antibiotics: linezolid, vancomycin and nitrofurantoin;
- 71.43% of the isolates proved methicillin-resistant;
- penicillin was active in only 30% of the cases;
- a high activity of clindamycin: 90.9%;
- a lower activity for macrolides: 40%.

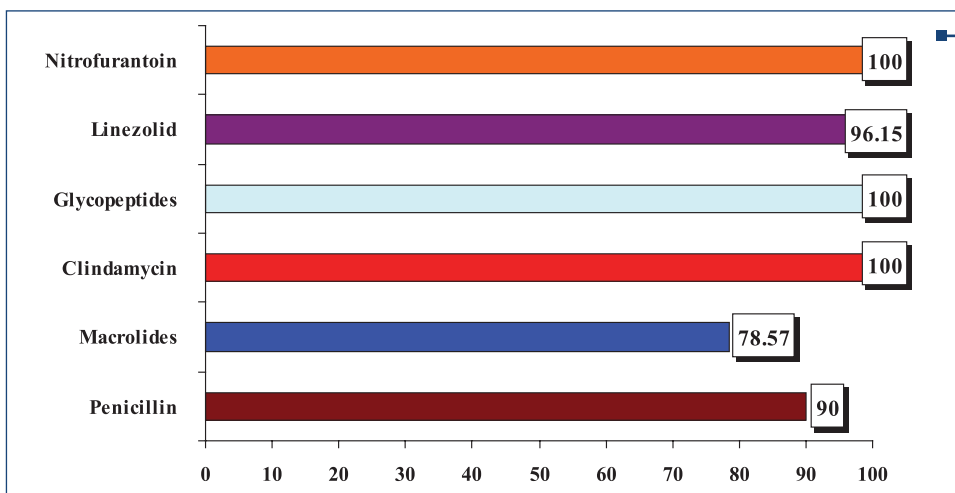


Figure 9. Antimicrobial susceptibility of group B *Streptococcus* strains: clindamycin (100%), rifampin (100%), glycopeptides (100%), nitrofurantoin (100%), penicillin (95%), macrolides (78.57%), linezolid (96.15%)

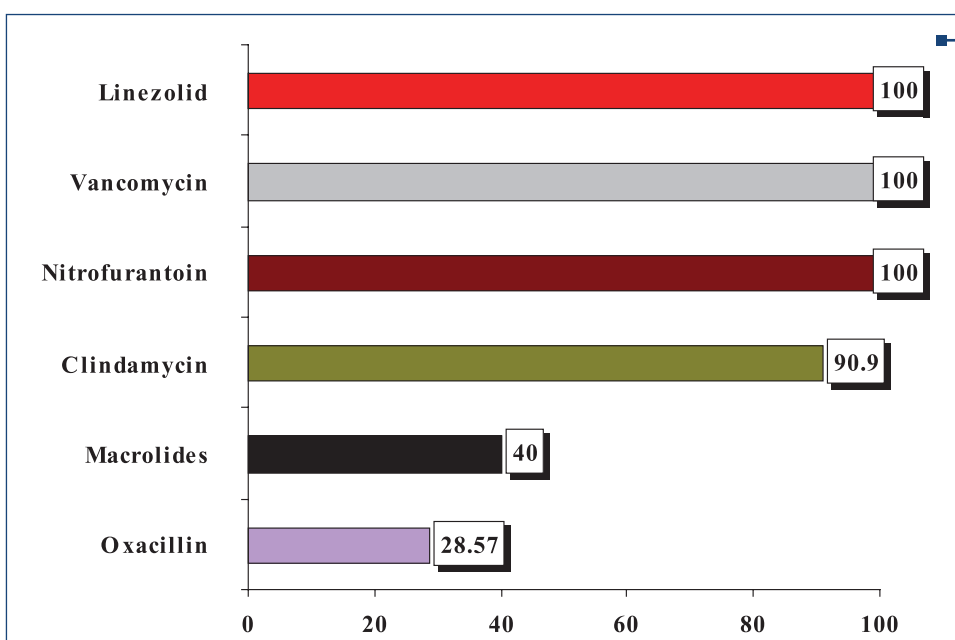


Figure 10. Antimicrobial susceptibility of group *Staphylococcus spp.* strains: linezolid (100%), vancomycin (100%), nitrofurantoin (100%), oxacillin (28.57%), clindamycin (90.9%), macrolides (40%)

Discussions

The analysis of germs isolated from urinary tract infections in women allows the updating of first line therapy recommendations in these cases, according to the most frequent clinical situations:

a. Asymptomatic bacteriuria

This usually does not require treatment, with three exceptions: pregnant women, patients with diabetes mellitus and preoperative, in the case of patients who will undergo urologic interventions.

Because this syndrome implies a bacterial identification, the treatment could be started with in vitro active antibiotics. Considering the high rate of *Escherichia coli*, the emerging tendency of these species' resistance to ciprofloxacin and the necessity to limit the use of quinolones, the following antibiotics are considered useful: nitrofurantoin (very high efficiency level) and fosfomicin (with the advantage of single dosage).

b. Lower urinary tract infection

The aforementioned alternatives can also be used in the treatment of uncomplicated cases of lower urinary tract infections, at least for the first episodes. The administration of a beta-lactam in clinical situations which do not allow the administration of the previous alternatives can be taken into account (for instance, in the case of pregnant women with glucose-6-phosphate dehydrogenase deficit); the resistance data indicate cefuroxime, for three days, as an alternative (amino-penicillins associated with a betalactamase inhibitor show an in vitro activity 15-20% lower).

In the case of recurrent or „complicated” cystitis the therapy duration is the standard one, 5-7 days, and the recommended treatment in this case is nitrofurantoin.

Regarding the fungal colony identification in urine (most frequently, *Candida spp.*) it represents, most often, a colonization which does not impose systemic antifungal treatment.

c. Upper urinary tract infections

In the case of low-severity, community-acquired upper urinary tract infections, a parenteral antibiotic should be used. This antibiotic should be effective against most of the possible involved etiologic agents (a very good activity against *Escherichia coli* is considered enough); the comparative analysis of *in vitro* efficiency indicates a superior activity of 3rd generation cephalosporins, compared to that of fluoroquinolones. Thus, ceftriaxone is recommended for an average of 7 days.

High-severity community, or hospital-acquired upper urinary tract infections impose the use of an active antibiotic therapy against the strains with particular resistance problems (multi-resistant *Enterobacteriaceae*, *Pseudomonas aeruginosa*); considering the low rate of ESBL-positive strains and the objective of maintaining carbapenems and colistin as second-line solutions, it is recommended to administer cefepime or piperacillin-tazobactam for 7-10 days; the association with an aminoglycoside, although frequently recommended, does not bring a certain, proved benefit. In these severe cases, the failure of the initial therapy should determine the following modifications:

- in the case of severe community-acquired infections, bacterial failure could indicate an ESBL-positive *Enterobacteriaceae* infection. In this case, the best option is to use a carbapenem: ertapenem, considering that community-acquired infections with non-fermentative gram negative bacilli are very rare. This allows us to avoid the use of active carbapenems, such as imipenem, meropenem, doripenem;

- *Pseudomonas aeruginosa* strains can be involved in nosocomial infections; this is why the only in vitro efficient option is colistin.

Conclusions

Resistance problems were recorded in the case of *Enterobacteriaceae*. The preliminary results show the novelty in antimicrobial treatment recommendations in pregnancy, which support the next issues:

- penicillins can no longer constitute an optimal therapeutic alternative, due to the low activity level. The association with a betalactamase inhibitor restores only partially the effect of aminopenicillins. Piperacillin-tazobactam proved to be the strongest of these associations;

- the presence of strains with low susceptibility or resistant to carbapenems could indicate testing errors. The reevaluation of these strains with CMI determination should be mandatory, in order to allow the observation of possible carbapenem-resistant *E. coli* clones. This represents a major public health issue and has an important role in nosocomial infection control;

- non-fermentative bacteria recorded high resistance levels for all the tested antibiotics; colistin proved to be the most efficient;

- for *Enterococcus faecalis*, ampicillin preserves a very good activity, while *Enterococcus faecium* strains are often multidrug-resistant; identifying isolates with reduced susceptibility to linezolid requires MIC confirmation;

- penicillins remain an effective alternative for the treatment of urinary infections caused by group *B streptococci*, but other antibiotics like nitrofurantoin showed very good activity and they are useful in patients with penicillin allergy;

- in the case of *Staphylococcus spp.* urinary tract infections, along with glycopeptides and linezolid, nitrofurantoin also proved a high activity level. ■

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