

## STATISTICAL STUDY ON THE BACTERIAL SENSIBILITY TESTED IN A SANITARY MICROBIOLOGY LABORATORY

CARMEN ȘERBAN<sup>1</sup>, NICULINA MITREA<sup>1</sup>, VIOREL ORDEANU<sup>2\*</sup>

<sup>1</sup>*University of Medicine and Pharmacy "Carol Davila" Bucharest, Romania*

<sup>2</sup>*Military Medical Research Center, Bucharest, Romania*

*\*corresponding author: ordeanu\_viorel@yahoo.com*

### Abstract

Hospital sanitary microbiology laboratories perform the sanitary inspection, especially in high-risk wards, control hospital flora contamination, which is often multiresistant, perform the periodic monitoring of staff at risk and the examination of persons outside the institution. Data on chemotherapy, germs and tested samples from a sanitary microbiology laboratory revealed specific issues. The database we acquired includes 35 antibiotics, 13 bacterial strains and 13 types of samples, totaling 43,892 bacterial sensibility tests. In statistical terms, only four antibiotics were effective (over 50%), but they are rarely used in therapy. For all the other antibiotics, resistant and intermediate results have prevailed. Pathogenic flora isolated, identified and tested in the hospital is generally multiresistant and creates the risk of nosocomial infections. The result of *in vitro* testing of bacterial sensibility to antibiotics is not always consistent with the *in vivo* results of antibiotherapy, the correlation is mainly statistical, but offers one effective indicator for prescribing antibacterial therapy.

### Rezumat

Laboratoarele de microbiologie sanitară din spital efectuează controlul igienico-sanitar, în special în secțiile cu risc înalt, contaminarea cu flora de spital, care adesea este polifarmacorezistentă, controlul periodic al personalului de risc și examinări pentru persoane din afara instituției. Datele referitoare la chimioterapicele, germenii și prelevatele testate din eșantionul statistic provenit din laboratoarele de microbiologie sanitară au relevat aspecte specifice. Banca de date achiziționate de noi cuprinde 35 de antibiotice, 13 specii bacteriene și 13 tipuri de prelevate, totalizând 43.892 de teste de sensibilitate bacteriană. Din punct de vedere statistic au fost eficiente (peste 50%) numai patru antibiotice, dar care sunt rareori utilizate în antibioterapie. La toate celelalte antibiotice au predominat rezultatele „Rezistent” și „Intermediar”. Flora patogenă izolată, identificată și testată în spital este în general polifarmacorezistentă și creează riscul de infecții nozocomiale. Rezultatul testării *in vitro* a sensibilității bacteriene la antibiotice nu concordă întotdeauna cu rezultatul *in vivo* al antibioterapiei; corelația este în principal statistică, dar oferă un indicator practic pentru prescrierea terapiei antiinfecțioase.

**Keywords:** antibiotics, bacterial resistance, antibiogram, hospital flora, antibiotic therapy

## Introduction

We investigated the *in vivo* bacterial sensibility testing data recorded by the sanitary microbiology laboratory of a clinical hospital in Bucharest, that doesn't have a department of infectious diseases, considered as representative for Romania. The hospital sanitary microbiology laboratory performs the sanitary inspection, especially in high-risk wards, controls hospital flora contamination, which is often multiresistant, performs the periodic monitoring of staff at risk and the examination of persons outside the institution. Data on chemotherapics, germs and tested samples from the sanitary microbiology laboratory revealed specific aspects [2,3,4,5,12,13,14].

## Materials and Methods

The database we created includes random results achieved during 2000-2010 and allows the acquisition, storage, retrieval, filtering and processing data. The antibiograms were performed on Mueller-Hinton agar culture medium, seeded with microbial suspension of microbial strain culture from patients or the environment, in 10 cm in diameter Petri dishes, aerobically incubated for 24 h, according to the technical recommendation for *in vitro* sensibility testing [6,7,8,10,15,16]. The reading was performed by measuring the diameter of inhibition zone in millimeters and reporting to the Susceptible, Intermediate, Resistant (SIR) system interpretation table.

The loaded database totals 43,892 sensibility tests, but we have eliminated those that are not in accordance with the recommendations in force, so we retained for statistical processing 38,756 tests. The laboratory performed different tests on the cocci and bacilli, gram-positive cocci and gram-negative bacilli. The antibiotics used were in total 35, of which 25 were used more frequently and in a standardized way (25,655 tests), as recommended by the World Health Organisation (WHO) and the Ministry of Health (MH)/Romanian Academy of Medical Sciences (RASM). Specific reagents were used, provided by: Labormed, Oxoid and Difco. Sometimes there were used antibiogram reagents for antibiotic sensibility testing, made of empirically standardized antibiotics, contrary to the recommendations, but with useful results to guide clinicians. Thus, the reagents were improvised in the laboratory from substances derived from quality pharmaceuticals, diluted with sterile starch and placed with microspatula as powder on the Mueller-Hinton agar medium Petri dish, seeded with microbial suspension of the bacterial strain isolated from

patients or the environment [1,9,11,17]. The number of bacterial species/groups isolated, identified and tested was 13, out of a total of 24 entities isolated and tested by antibiogram (ABG).

Note that the recommendations for antibiotigram are mandatory for clinical microbiology laboratories, but are only indicative for other microbiology laboratories. It should be noted that, out of the total pathogen sensibility tests made in the sanitary microbiology laboratory, only a part follow the recommendations for clinical antibiogram, the rest being tested according to specific criteria set out in the laboratory. The difference between the share of different groups of isolated, identified and tested bacteria in laboratories as a result of differential use of data: the treatment of patients (Clinical Microbiology Laboratory) and estimation of hospital flora contamination (Sanitary Microbiology Laboratory). Of all isolated, identified and tested bacteria in medical microbiology laboratories (clinical and sanitary) in the first place is *Escherichia coli*, respectively 49.45%, the prevalence for clinical microbiology is 50.95% (absolute majority) and for the sanitary microbiology 38.86% (majority). We recall that in May 2011 an epidemic of pathogen *E. coli* broke in the E.U., with hundreds of cases hospitalized and dozens of deaths. Given that each healthy individual hosts in the digestive tract billions saprophyte *E. coli*, results the spread and medical importance of these enterobacteria, the risk of contamination and the difficulty of differentiating pathogenic strains of the saprophytic. As a result, it was expected that this bacteria to be most frequently isolated and identified in the medical microbiology laboratories, especially those of clinical microbiology. Samples from sanitary microbiology laboratory were 2,086 totaling 13 types, the most common being the eye discharge, the nasal exudate and the pharyngeal exudate.

### Results and Discussion

We performed the statistical processing of medical and pharmaceutical interpretation of data for bacterial sensibility to antibacterial agents in the sanitary microbiology laboratory. It is known that the result of *in vitro* testing of bacterial sensibility to antibiotics is not always consistent with the *in vivo* results of the antibiotic treatment. The correlation is mainly statistical, but offers the most effective indicator for prescribing anti-infective therapy.

The 13 types of microbiological samples allowed the isolation, identification and antibiogram testing of 13 bacteria. Bacterial sensitivities

were tested to 35 antibiotics, but because some were not standardized, we considered only 25 of them. There were removed from the statistical processing the results of sensibility tests made on a non-standard procedure in the laboratory, without complying with the recommendations. Those antibiotics for which the number of tests was below 10 were excluded from the statistical processing because they were unreliable statistically, being insufficient, e.g. carbenicillin, being present in a few cases, was not included in the statistical processing. It is not surprising that the first 3 antibiotics, neomycin, novobiocin and colistin, are the most effective in the antibiogram, because are rarely used in therapy.

The overall sensibility of the studied group is very good only for an antibiotic (over 66.66%), is good for 9 antibiotics (between 33.33% and 66.66%) and poor for the other 25 antibiotics (under 33.33%). As e.g. Augmentin<sup>®</sup> has the highest rank of resistance, so the corresponding antibiotic appears to be compromised for therapy, as sulfafurazol, which is the oldest antibacterial chemotherapy, introduced in medical practice in the '30s. The number of intermediate results is very high, which indicates either systematic technical and interpretation errors or overall devaluation of the antibiotic by reducing the sensibility. The medical practice considers that the most useful indication is Sensible and combines the result of Intermediate with Resistant, based on the therapeutic effect. As a result, we have considered two cases: Sensible and Resistant, the last also comprising the Intermediate results.

Testing sensibility to 35 antibiotics totals 38,576 tests, of which 25,656 standardized tests with a test average of 733 (Table I). Note the high percentage of tests with non-standardized reagents, prepared in the laboratory, the results being only indicative and are not included in our statistical processing.

From the data presented in table I, results that reagents were also used inconsistent to WHO/MH recommendations, using empirical standardized antibiotics, prepared from therapeutic drugs: Rocephin<sup>®</sup> (ceftriaxone), furazolidone, gentamicin, Zinacef<sup>®</sup> (cefuroxime), doxycycline, Keflin<sup>®</sup> (cephalothin), azithromycin, streptomycin, Fanasil<sup>®</sup> (sulfadoxine). We consider that the results obtained are not conclusive, are indicative, but useful for the medical practitioner, at screening. This demonstrates the need for permanent updating of the range of antibacterial chemotherapy used for antibiograms, according to the medical and pharmaceutical practice.

**Table I**

Frequency of use in testing of standardized antimicrobial chemotherapy in sanitary microbiology laboratory

| No. | Antimicrobial chemotherapy |     | Test no.      | %      | Rank      | Observations |
|-----|----------------------------|-----|---------------|--------|-----------|--------------|
| 1.  | Rifampicin                 | R   | 1899          | 7.40   | <b>1</b>  | frequent     |
| 2.  | Co-trimoxazole             | SxT | 1796          | 7.00   | <b>2</b>  | frequent     |
| 3.  | Oxacillin                  | O   | 1770          | 6.90   | <b>3</b>  | frequent     |
| 4.  | Kanamycin                  | K   | 1719          | 6.70   | <b>4</b>  | frequent     |
| 5.  | Erythromycin               | E   | 1695          | 6.61   | <b>5</b>  | frequent     |
| 6.  | Colistin                   | Co  | 1683          | 6.56   | <b>6</b>  | frequent     |
| 7.  | Tetracycline               | T   | 1676          | 6.53   | <b>7</b>  | frequent     |
| 8.  | Chloramphenicol            | C   | 1659          | 6.47   | <b>8</b>  | frequent     |
| 9.  | Augmentin                  | Au  | 1580          | 6.16   | <b>9</b>  | frequent     |
| 10. | Gentamicin                 | G   | 1508          | 5.88   | <b>10</b> | frequent     |
| 11. | Amoxicillin                | Ax  | 1442          | 5.62   | <b>11</b> | frequent     |
| 12. | Penicillin G               | P   | 1406          | 5.48   | <b>12</b> | frequent     |
| 13. | Streptomycin               | S   | 1337          | 5.21   | <b>13</b> | frequent     |
| 14. | Ampicillin                 | A   | 1193          | 4.65   | <b>14</b> | frequent     |
| 15. | Lincomycin                 | Li  | 1012          | 3.94   | <b>15</b> | frequent     |
| 16. | Neomycin                   | N   | 359           | 1.40   | <b>16</b> | rare         |
| 17. | Cephalothin                | Cf  | 337           | 1.31   | <b>17</b> | rare         |
| 18. | Doxycycline                | Do  | 311           | 1.21   | <b>18</b> | rare         |
| 19. | Novobiocin                 | No  | 287           | 1.12   | <b>19</b> | rare         |
| 20. | Nitrofurantoin             | Nf  | 260           | 1.01   | <b>20</b> | rare         |
| 21. | Nalidixic acid             | Nx  | 251           | 0.98   | <b>21</b> | rare         |
| 22. | Furazolidone               | Fu  | 204           | 0.80   | <b>22</b> | rare         |
| 23. | Sulfafurazole              | Su  | 203           | 0.79   | <b>23</b> | rare         |
| 24. | Polymyxin                  | Po  | 54            | 0.21   | <b>24</b> | very rare    |
| 25. | Carbenicillin              | Cb  | 14            | 0.05   | <b>25</b> | very rare    |
|     | <b>TOTAL</b>               |     | <b>25,655</b> | 100.00 |           |              |

Isolated and identified pathogenic bacteria comprise in 13 species (Table II).

**Table II**

The frequency and rank of isolated, identified and tested for antibiogram bacteria in the sanitary microbiology laboratory

| No. | Isolated germ                        | No. of tested strains | %      | Rank      | Observations            |
|-----|--------------------------------------|-----------------------|--------|-----------|-------------------------|
| 1.  | <i>Escherichia coli</i>              | 82                    | 38.86  | <b>1</b>  | Isolated and identified |
| 2.  | <i>Proteus</i> sp.                   | 33                    | 15.64  | <b>2</b>  | Isolated and identified |
| 3.  | <i>Bacillus cereus</i>               | 19                    | 9.00   | <b>3</b>  | Isolated and identified |
| 4.  | <i>Staphylococcus saprophyticus</i>  | 18                    | 8.53   | <b>4</b>  | Isolated and identified |
| 5.  | <i>Enterobacter</i> sp.              | 15                    | 7.11   | <b>5</b>  | Isolated and identified |
| 6.  | <i>Streptococcus beta-hemolytic</i>  | 15                    | 7.11   | <b>5</b>  | Isolated and identified |
| 7.  | <i>Staphylococcus aureus</i>         | 10                    | 4.74   | <b>6</b>  | Isolated and identified |
| 8.  | <i>Pseudomonas aeruginosa</i>        | 6                     | 2.84   | <b>7</b>  | Isolated and identified |
| 9.  | <i>Streptococcus pyogenes grup A</i> | 6                     | 2.84   | <b>7</b>  | Isolated and identified |
| 10. | <i>Streptococcus viridans</i>        | 3                     | 1.42   | <b>8</b>  | Isolated and identified |
| 11. | <i>Streptococcus</i> sp.             | 2                     | 0.95   | <b>9</b>  | Isolated and identified |
| 12. | <i>Citrobacter</i> sp.               | 1                     | 0.47   | <b>10</b> | Isolated and identified |
| 13. | <i>Klebsiella</i> sp.                | 1                     | 0.47   | <b>10</b> | Isolated and identified |
|     | <b>TOTAL</b>                         | <b>211</b>            | 100.00 |           |                         |

The number of tests made per antibiotic is variable and the results are generally good, which shows a differentiated and judicious choice to perform the test. Of the 35 tested antibiotics, one had a very good effect neomycin (85.24%), 9 had a good effect (between 33.81 and 57.14%) and 25 had little effect (less than 33.33%). Of antibiotics with little effect, the overall most ineffective were: lincomycin (6.32%) and five non-standard antibiotics (Rocephin<sup>®</sup>, gentamicin, Zinacef<sup>®</sup>, Keflin<sup>®</sup>, streptomycin), thus confirming statistically the recommendation that the analysis should use only standardized reagents. We consider that in this case there was a systematic error in the laboratory either from the underdosing or by improper storage of the prepared reagent. Recalling the requirements of good laboratory practice, the materials used should be standardized, to carry out internal quality control and to participate in external quality controls. The test results are as expected, according to the current trends of bacterial resistance and antibiotic sensibility. There were 13 samples from which the test bacteria were grown (Table III).

**Table III**

Frequency of types of samples for antibiograms in the sanitary microbiology laboratory

| No.          | Analysed microbiological samples | Sample no.   | %             | Rank      | Observations |
|--------------|----------------------------------|--------------|---------------|-----------|--------------|
| 1.           | Purulent eye discharge           | 948          | 45.45         | <b>1</b>  | frequent     |
| 2.           | Nasal exudate                    | 541          | 25.93         | <b>2</b>  | frequent     |
| 3.           | Pharyngeal exudate               | 361          | 17.31         | <b>3</b>  | frequent     |
| 4.           | Urine culture                    | 94           | 4.51          | <b>4</b>  | rare         |
| 5.           | Purulent ear discharge           | 52           | 2.49          | <b>5</b>  | rare         |
| 6.           | Purulent vaginal discharge       | 19           | 0.91          | <b>6</b>  | rare         |
| 7.           | Wound secretion                  | 17           | 0.81          | <b>7</b>  | rare         |
| 8.           | Pus boil                         | 16           | 0.77          | <b>8</b>  | rare         |
| 9.           | Stool                            | 12           | 0.58          | <b>9</b>  | rare         |
| 10.          | Purulent urethral discharge      | 10           | 0.48          | <b>10</b> | rare         |
| 11.          | <i>Others</i>                    | 16           | 0.77          | -         | rare         |
| <b>TOTAL</b> |                                  | <b>2,086</b> | <b>100.00</b> |           |              |

**Pharyngeal exudate.** The bacterial sensibility was tested for 35 antibiotics, but because some were not standardized we considered only 25 of them. There were removed from the statistical processing the results of sensibility tests that were made on a non-standard procedure in laboratory, without complying with the recommendations. Of the 25 antibiotics there were tested 19, totaling 4,714 tests with an average of 248.10 tests/antibiotic. Number of tests/antibiotic: minimum 52 and maximum 354. The sensibility was not good at any of the tested antibiotics. High resistance was recorded to 16 antibiotics: lincomycin, novobiocin (90%), rifampicin, carbenicillin, gentamicin, colistin, ampicillin, penicillin (80%), oxacillin,

nitrofurantoin, furazolidone, doxycycline, chloramphenicol, amoxicillin, streptomycin, tetracycline (70%). We consider it inappropriate to test for furazolidone the pharyngeal exudate. Bacteria tested were: staphylococci, streptococci, *Bacillus cereus*, and gram-negative bacteria *Proteus* sp. It was not made a differentiated statistical analysis of bacterial species, because the number of data available for each of them is not statistically significant. Hemolytic staphylococci and streptococci from the pharyngeal exudate were most frequent pathogens identified and tested in this laboratory during the studied period.

**Nasal exudate.** 22 antibiotics were tested, of which 20 frequently, totaling 4,902 tests with an average of 245.10 tests/antibiotic. We consider that there is inadequate to test furazolidone for the nasal exudate (365 cases). Number of tests/antibiotic: minimum 60 and maximum 369. Sensibility was not good at any of the tested antibiotics. High resistance was recorded to 16 antibiotics: lincomycin, oxacillin, sulfafurazol (over 90%), amoxicillin, nalidixic acid, augmentin, erythromycin, kanamycin and rifampicin (over 80%), streptomycin, polymyxin, chloramphenicol, gentamicin, tetracycline, penicillin, co-trimoxazole (over 70%). The tested bacteria were: staphylococci, hemolytic streptococci, *Bacillus cereus*, gram-negative bacilli, *Proteus*. A differentiated statistical analysis of the bacterial species was not performed, because the number of data available for each of them is not statistically significant.

**Purulent nasal discharge.** Of the 35 antibiotics there were tested 20, totaling 2,121 tests with an average of 111.63 tests/antibiotic, minimum 11 and maximum 166. The strain sensibility was not good at any of the antibiotics that were tested. High resistance was recorded to 15 antibiotics: oxacillin, lincomycin, amoxicillin (over 90%), nalidixic acid, erythromycin, rifampicin, Augmentin<sup>®</sup>, streptomycin and sulfafurazol (over 80%), kanamycin, co-trimoxazole, polymyxin, chloramphenicol, gentamicin and tetracycline (over 70%).

**Purulent eye discharge.** Of the 25 antibiotics 21 were tested, of which 20 were tested and statistically processed, totaling 12,620 tests with an average of 631 tests/antibiotic. We consider it inappropriate to test aminoglycosides (neomycin, gentamicin, streptomycin, kanamycin) for purulent eye discharge, because aminoglycosides topical ocular applications should be avoided because it can cause local irritation [1], and furazolidone, which is a yellow dye that can lead to false diagnosis of jaundice. Number of tests/antibiotic: minimum 33 and maximum 940. The sensibility was not good at any of the tested antibiotics. High resistance has been recorded to 16 antibiotics: oxacillin, lincomycin, amoxicillin (over 90%), erythromycin,

rifampicin, sulfafurazol, augmentin, nalidixic acid, gentamicin (over 80%), streptomycin, co-trimoxazole, kanamycin, tetracycline, polymyxin, chloramphenicol, ampicillin (over 70%). The tested bacteria were: *B. cereus*, *E. coli*, *Enterobacter* sp., hemolytic flora, gram-negative bacilli, *Proteus* sp., *S. aureus*, *S. saprophiticus*.

**Purulent ear discharge.** Of the 36 antibiotics 15 were tested and statistically processed, totaling 632 tests with an average of 42.13 tests/antibiotic, the number of tests/antibiotic: minimum 24 and maximum 52. The sensibility was not good at any of the tested antibiotics. High resistance was recorded to 11 antibiotics: lincomycin, augmentin (over 90%), erythromycin, oxacillin, rifampicin, amoxicillin, streptomycin and penicillin (over 80%), co-trimoxazole, chloramphenicol, kanamycin (over 70%). The tested bacteria were: staphylococci and gram-negative bacilli, but no species was sufficient for statistical processing.

**Pus boil.** Of the 25 antibiotics 18 were tested and statistically processed, of which 12 frequently, totaling 175 tests with an average of 14.58 tests/antibiotic. Number of tests/antibiotic: minimum 13 and maximum 15. The sensibility was not good at any of the tested antibiotics. High resistance was recorded at 8 antibiotics: erythromycin, oxacillin and chloramphenicol (over 90%), co-trimoxazole, augmentin, kanamycin (over 80%), streptomycin, amoxicillin (over 70%). It was not made a differentiated statistical analysis of bacterial species, because the number of data available for each of them was not statistically significant.

**Purulent urethral discharge.** Of the 35 antibiotics 5 were tested and statistically processed, totaling 50 tests with an average of 10 tests/antibiotic, number 10. The sensibility was not good at any of the tested antibiotics. Resistance occurred in five antibiotics: colistin, tetracycline (100%), rifampicin (over 80%), kanamycin, augmentin (over 70%). The tested bacteria were gram-positive cocci and gram-negative bacilli, but no species was large enough for statistical processing.

**Purulent vaginal discharge.** Of the 35 antibiotics 15 were tested and statistically processed, totaling 246 tests with an average of 16.40 tests/antibiotic, minimum 12 and maximum 19. The sensibility was not good at any of the tested antibiotics. Resistance was recorded to 11 antibiotics: oxacillin, lincomycin (over 90%), augmentin, erythromycin, amoxicillin, rifampicin, gentamicin, kanamycin (over 80%), co-trimoxazole, tetracycline and chloramphenicol (over 70%). The tested germs were gram-positive cocci and gram-negative bacilli, but no species was large enough for statistical processing.

**Urine culture.** Of the 35 antibiotics 19 were tested and statistically processed, totaling 1211 tests with an average of 63.74 tests/antibiotic with minimum 17 and maximum 93. The sensibility was not good at any of the tested antibiotics. Resistance was recorded to 14 antibiotics: amoxicillin, lincomycin, oxacillin (over 90%), nalidixic acid, erythromycin, kanamycin, sulfafurazol (over 80%), furazolidone, penicillin, tetracycline, chloramphenicol, co-trimoxazole, ampicillin, colistin (over 70%). The tested germs were gram-positive cocci and gram-negative bacilli, but no species was large enough for statistical processing.

From the gram-negative bacilli, most were *E. coli*, other *Enterobacteriaceae* being insufficient for statistical processing. *E. coli* was tested on 14 antibiotics of 35, totaling 601 tests with an average of 42.93 tests/antibiotic, minimum 29 and maximum 49. The sensibility was not good at any of the tested antibiotics. Resistance was recorded to seven antibiotics: kanamycin (over 90%); sulfafurazol, chloramphenicol, nalidixic acid (over 80%), co-trimoxazole, tetracycline, furazolidone (over 70%).

**Stool.** Of the 25 antibiotics 21 were tested and statistically analysed, of which 10 frequently, totaling 109 tests with an average of 10.90 tests/antibiotic. It is noted that sulfafurazol and streptomycin were rarely tested, although they are often recommended to treat diarrheal diseases. The sensibility was not very good at any of the tested antibiotics. High resistance was recorded to three antibiotics: tetracycline (90%), kanamycin (80%), colistin (70%). The most frequently identified pathogen bacteria was *E. coli*, but the insufficient number of tests did not allow the statistical processing.

The phenomenon of bacterial resistance to antibiotics, sanitary microbiology laboratory observed is according with bacterial resistance observed in clinical microbiology laboratory of the same hospital.

### Conclusions

The database acquired from a sanitary microbiology laboratory includes 35 antibiotics, 13 bacterial species and 13 types of samples, totaling 43,892 bacterial sensibility tests. The share of the identified bacterial strains for microbiological diagnosis is very low compared to international reports, which shows impaired identification in the laboratory. The number of tests / antibiotic is variable and the results are relatively good, which shows a differentiated and judicious choice of most tests. In statistical terms, only four antibiotics were effective: Neomycin, Carbenicillin, Novobiocin and Colistin (over 50%), but they are rarely used in therapy, so the result has no practical importance. At all the other 31

antibiotics resistant and intermediate results have prevailed, so pathogenic flora isolated, identified and tested in the hospital is generally multiresistant and creates the risk of nosocomial infections.

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