Monitoring of antibiotic resistance in hospital isolates at the Clinical Center in Niš

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Predrag Stojanovic, Vojislav Ciric

Serbia
ANTIMICROBIC DRUGS

OUR FAILURE ?
Bacteria will develop resistance to every kind of antibiotic if they are given enough time and enough antibiotics.

Stuart B. Levy
In their fight for the survival, bacteria have undreamt genetic possibilities.

Trying to protect from them, a man uses its experience, knowledge, intelligence and huge resources as possible sources of new antimicrobial medicines.

The final outcome of this fight will be the speed.
Are we ready and quick enough in discovering new kinds of antibiotics?

Or maybe we should consider all the other possibilities for preventing the resistance and its development.

And all this is for those who are about to be born and grow and to whom we have the responsibility not to leave them without the possibility to defend against the infections.
Distribution of penicillin resistant *S. pneumoniae* in Europe
There are two main reasons!
Resistance (R) bacteria

Susceptibility (S) to antimicrobial drugs
Improving diagnosis in the microbiology laboratory

- Isolate VRE Colonies
- Isolate MRSA Colonies
- Isolate ESBL Colonies
- Isolate Penicillin resistance *Strep. pneumoniae* Colonies
Generally high resistance level of bacteria in our geographical area!

- Clinical picture gravity
- The low rate of the success of therapy
- The possibility of spreading of the resistant isolates in a hospital environment
- Pharmacoeconomic approach

The problem of special importance!
Monitoring parameters of bacterial isolates

- Type of bacteria
- Type of inpatient material
- Clinic from which the isolate has been obtained
- Sensitivity/Resistance (S/R) to antimicrobial drugs and/or MIK
Detection methodology of antimicrobial sensitivity

- Disc diffusion method
- Dilution method - agar dilution method
- Combined method (E – EPSILON test)
All test results have been processed in:

Acces, Excel and EPI 5
Antimicrobial Susceptibility Testing is in accordance with CLSI (NCCLS) protocol

„Rosco Diagnostica“

E test „AB Biodisc“
<table>
<thead>
<tr>
<th>LEK</th>
<th>REZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP</td>
<td>S</td>
</tr>
<tr>
<td>AMK</td>
<td>S</td>
</tr>
<tr>
<td>NA</td>
<td>S</td>
</tr>
<tr>
<td>CC</td>
<td>S</td>
</tr>
<tr>
<td>DOX</td>
<td>R</td>
</tr>
<tr>
<td>H</td>
<td>S</td>
</tr>
<tr>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>G</td>
<td>S</td>
</tr>
</tbody>
</table>

**Record:** 1 of 8
Inpatient sample material

2769

31442

- Negative
- Positive
Number of positive isolates from the clinics within Clinical Center in Niš
The most common bacteria isolated in CC Nis
(of 2769 isolates)

- **E.coli**:
  - Isolates: 449

- **Staph.epidermidis**:
  - Isolates: 266

- **Staph.spp.**:
  - Isolates: 215

- **Enterobacter spp.**:
  - Isolates: 187

- **Klebsiella spp.**:
  - Isolates: 153

- **S.pneumoniae**:
  - Isolates: 106

- **Haemophilus spp.**:
  - Isolates: 55

- **Others**:
  - Isolates: 262

- None of the above listed bacteria were isolated.
The data regarding the overall sensitivity to the antimicrobial drugs
E. coli – basic and extended antibiogram
**Escherichia coli**

(multi-resistant isolates and resistance to cephalosporin)

- Ceftazidim 66%
- Cefepim 54%
Comparable results between *E. coli* and *Enterobacter spp.*
- basic and extended antibiogram -
Comparative resistance between *Staphylococcus aureus* and *Staphylococcus epidermidis* to antimicrobics drugs.
*Staphylococcus aureus* and *S. epidermidis* (675 isolates) show similar profiles of resistance:

- Penicillin 97% vs. 93% $c^2=3.05$, $p=0.080$; n.s.
- Oxacillin 49% vs. 67% $c^2=21.33$, $p<0.001$; s.z. OR=2.12 95% CI 1.5-3.0
- Erytromycin 40% vs. 50%
- Clindamycin 33% vs. 39%
- Ofloxacin 30%
- Vankomycin 0%
Staphylococcus aureus (MRSA)
Comparative resistance between *Pseudomonas aeruginosa* and *Acinetobacter spp.*
**Pseudomonas aeruginosa**
- show the highest level of resistance to tested antimicrobial drugs

<table>
<thead>
<tr>
<th>Combination</th>
<th>Resistant (%)</th>
<th>$\chi^2$</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTR i CAZ</td>
<td>85%-56%</td>
<td>46,54</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td></td>
<td>OR=4,59 (95%CI 2,8-7,4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAZ i I</td>
<td>56%-7%</td>
<td>125,34</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td></td>
<td>OR=16,90 (95%CI 9,2-31,3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAZ i MER</td>
<td>56%-14%</td>
<td>78,21</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td></td>
<td>OR=7,27 (95%CI 4,5-11,9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G i A</td>
<td>75%-49%</td>
<td>31,62</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td></td>
<td>OR=3,09 (95%CI 2,0-4,7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The most frequent invasive isolates:

CSF
- Staphylococcus epidermidis
- Staphylococcus aureus
- Staphylococcus spp.
- Streptococcus pneumoniae
- Acinetobacter spp.
- Haemophylus influenzae
- Enterobacter spp.

BLOOD CULTURE
- Staphylococcus epidermidis
- Staphylococcus aureus
- Staphylococcus spp.
- Pseudomonas aeruginosa
- Enterobacter spp.
- Enterococcus faecalis
- Acinetobacter spp.
Inpatient sample materials and resistant isolates frequency

Isolates

- B. rane
- B. ven. kat.
- B. tubusa
- Per.tecn.
- Hemokulture
- Likvor

% R | % S
---|---
0% | 20% | 40% | 60% | 80% | 100%

Legend:
- % R
- % S
*Pseudomonas aeruginosa* – isolates from the Pediatric Clinic in comparison to other isolates from the CC Nis (49 / 204)
Infections with multiresistant Gram-positive and Gram-negative pathogens have become a great and urgent clinical and therapeutic problem. Patients in ICU, haematological and transplantation departments have special risks for these infections and encounter high mortalities.

Optimising prevention, diagnostic measurements and appropriate therapeutic decisions based on pathogenetic insights is a big challenge for physicians who are responsible for these patients.
Enterococci are among the most frequent carriers of hospital-acquired infections, especially in the intensive care units. They may cause meningitis and bacteriemia in newborn infants.

The frequency of the resistance of the Enterococcus faecalis isolates:
Growing bacterial resistance means that what were once effective and cheap treatments for infections caused by Gram-positives have now been lost, including penicillin and - in hospitals - oxacillin for use against staphylococcal infections. Mortality is increased among ICU patients where infections are resistant to first- and second-line empirical therapies. The presence of multidrug-resistant (MDR) Gram-positive bacteria has been associated with increased rates of re-operation, surgical-site infection and abscess formation in intra-abdominal infections. In the specific case of MRSA, outcomes are worse and costs higher for patients with infections due to these strains. Vancomycin-resistant enterococci (VRE) are also increasing in number in many European hospitals and constitute a major therapeutic challenge for clinicians. As a whole, multiresistant Gram-positive pathogens have become an urgent and sometimes unmanageable problem in the ICU, as well as in pneumonology, oncology and urological wards.
Distribution of samples from which the tested enterococcus classes were isolated

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Urine</th>
<th>Blood</th>
<th>Wound smear</th>
<th>Vaginal smear</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococ. faecalis</td>
<td>71</td>
<td>6</td>
<td>15</td>
<td>20</td>
<td>112</td>
</tr>
</tbody>
</table>
Enterococcus faecalis (112 isolates) - resistance profile to tested antimicrobial drugs -

PEN  ER  AMP  DOX  VA

% R % S
**Resistance of Enterococ. faecalis to penicillin and ampicillin**

<table>
<thead>
<tr>
<th>Enterococcus faecalis</th>
<th>Penicillin</th>
<th>Ampicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of tested classes (112)</td>
<td>29 (25.89%)</td>
<td>6 (5.36%)</td>
</tr>
</tbody>
</table>

\[ X^2 = 17.83 \text{ for } p < 0.01 \]

All the tested enterococcus isolates (n = 112) were sensitive to vancomycin.

**High-level resistance of enterococci to aminoglycosides**

<table>
<thead>
<tr>
<th>Enterococcus faecalis</th>
<th>Gentamicin</th>
<th>Streptomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of tested classes (112)</td>
<td>57 (50.89%)</td>
<td>55 (50%)</td>
</tr>
</tbody>
</table>

The differences in resistance between gentamicin and streptomycin were statistically insignificant for P = 0.005.
### Resistance of Enterococcus faecalis classes to antibiotics

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetracycline (n = 112)</td>
<td>76 (67.86)</td>
</tr>
<tr>
<td>Chloramphenicol (n = 112)</td>
<td>82 (73.21)</td>
</tr>
<tr>
<td>Rifampin (n = 112)</td>
<td>75 (66.96)</td>
</tr>
<tr>
<td>Amoxicillin/clavulanic acid (n = 112)</td>
<td>6 (5.36)</td>
</tr>
<tr>
<td>Ciprofloxacin (n = 112)</td>
<td>31 (27.68)</td>
</tr>
<tr>
<td>Norfloxacin (n = 112)</td>
<td>62 (55.36)</td>
</tr>
</tbody>
</table>
There were no statistically significant differences between penicillin and ciprofloxacin, ampicillin and amoxicillin with clavulanic acid and gentamicin and norfloxacin.

As compared to penicillin, vancomycin performed a significantly greater efficiency ($X^2 = 33.16$ for $p < 0.01$), while the efficiency related to ampicillin was on the border of statistical significance ($X^2 = 6.14$ for $p < 0.01$). Ciprofloxacin showed significantly higher efficiency as compared to norfloxacin ($X^2 = 17.59$ for $p < 0.01$).

We registered the resistance of *Enterococcus faecalis* to chloramphenicol, tetracycline and rifampin.
Antimicrobial resistance of *Streptococcus pneumoniae* strains to penicillin and ceftriaxone, isolated in the Niš district during 1999-2006 and 2007.
The penicillin resistant strain of *S. pneumoniae* (PRSP)

- *Streptococcus pneumoniae* holds a prominent place among the causes of infections of the respiratory tract, along with those of the middle ear and central nervous system.
- *S. pneumoniae* has been isolated in around 30% of the etiologically verified acute respiratory infections (ARI) and is combined with a significantly high number of terminal cases.
- Globally speaking, *S. pneumoniae* is annually connected with the death of one million children under the age of 5.
• Up until the mid 1960’s all of the S. pneumoniae strains could be treated with penicillin (MIC<0.06 µg/ml),
• A resistance to penicillin was first registered in Boston in 1965, and in Australia in 1966.
• In our area the occurrence of strains resistant to penicillin was registered for the first time in 1977.
• The data from 1995 indicate a decreased sensitivity to penicillin and a high sensitivity to cephalosporin of the third generation.
• During 1996, an increase was noted in the resistance of hospital strains in comparison to the strains from the nose swab, as well as the existence of a higher rate of multi-drug resistant isolates (MDR) obtained from hospital materials.
The review of resistance moving on examined antibiotics with isolates of S. pneumoniae of the hospital origin – 2007.
SENSITIVITY OF S. PNEUMONIAE TO PENICILLIN
BY MEANS OF THE AGAR DILUTION METHOD
SENSITIVITY OF S. PNEUMONIAE TO CEPHTRIAXON
BY MEANS OF THE AGAR DILUTION METHOD

9,40%
10,00%
I - intermedijarni
R - rezistentni
S - senzitivni

80,60%
## S. PNEUMONIAE MULTIPLE RESISTANCE

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>R</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>OX + TS</td>
<td>683</td>
<td>31,26</td>
</tr>
<tr>
<td>OX + ER</td>
<td>398</td>
<td>18,22</td>
</tr>
<tr>
<td>OX + AZ</td>
<td>381</td>
<td>17,44</td>
</tr>
<tr>
<td>OX + T</td>
<td>361</td>
<td>16,52</td>
</tr>
<tr>
<td>OX + ER + TS</td>
<td>259</td>
<td>11,85</td>
</tr>
<tr>
<td>OX + AZ + TS</td>
<td>253</td>
<td>11,58</td>
</tr>
<tr>
<td>OX + AZ + T</td>
<td>214</td>
<td>9,79</td>
</tr>
<tr>
<td>OX + ER + T</td>
<td>213</td>
<td>9,75</td>
</tr>
<tr>
<td>OX + ER + TS + T</td>
<td>149</td>
<td>6,82</td>
</tr>
<tr>
<td>OX + AZ + TS + T</td>
<td>147</td>
<td>6,73</td>
</tr>
<tr>
<td>OX + ER + TS + L</td>
<td>121</td>
<td>5,54</td>
</tr>
<tr>
<td>OX + OF</td>
<td>90</td>
<td>4,12</td>
</tr>
<tr>
<td>OX + ER + T + L</td>
<td>60</td>
<td>2,75</td>
</tr>
<tr>
<td>OX + ER + T + OF</td>
<td>25</td>
<td>1,14</td>
</tr>
<tr>
<td>OX + ER + TS + T + OF</td>
<td>17</td>
<td>0,78</td>
</tr>
<tr>
<td>OX + ER + TS + OF + T + H + RIF</td>
<td>17</td>
<td>0,78</td>
</tr>
<tr>
<td>OX + LG</td>
<td>15</td>
<td>0,69</td>
</tr>
<tr>
<td>OX + ER + TS + OF + T + H + RIF + LI</td>
<td>3</td>
<td>0,14</td>
</tr>
</tbody>
</table>
Infection control

- At the beginning of the new millenium, a national inquiry indicated that the quality of organisation of infection control was quite variable between hospitals especially in institutions were infection control practitioners could only spend a small proportion of their professional time to infection control.
- The infection control team must investigate, develop and propose priorities for their hospital, determine the necessary resources, objectives, methods for development, implementation and follow up.
CLOSTRIDIUM DIFFICILE ASSOCIATED DISEASE IN PATIENTS HOSPITALIZED IN THE CLINICAL CENTAR NIS - SERBIA
“Clostridium difficile - Associated Disease: Underdiagnosed, Underreported, Undertreated. How to Overcome the Challenges”

- *Clostridium difficile* causes antibiotic-associated diarrhoea, colitis and pseudomembranous colitis.
- The emergence of the new virulent CD (PCR ribotype O27, PFGE type NAP1) that produces more toxin A and toxin B plus a binding toxin is found in the USA, Canada, and now in European countries.
Acurate Diagnosis and Testing for CDAD

- *Clostridium difficile* is now recognized as the primary cause of hospital-acquired colitis in patients who receive antibiotics...

- Molecular typing methods (PCR ribotyping, PFGE) help to follow the spread of *C. difficile* in the hospitals and community.
Quantification of Clostridium difficile by real-time PCR in hospital environmental samples

- The sites sampled comprised bed frames, commodes, toilet environment, patient side room, floors, staff and patient hands. 86 isolates (40.6%) recovered from the hospital environment were positive for the presence of C. difficile.
- The higher numbers of C. difficile being found in the hands of patients and staff, staff gloves and in the toilets.
- Considering the importance of staff and the inanimate hospital environment as a potential source of C. difficile, close attention should be paid to the hygiene of the clinical settings.
## The find of Clostridium difficile in examination group

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Number of samples</th>
<th>Number and percent of patients with find C. difficile</th>
<th>Number and percent of patients with find toxin in stool samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Clinical</td>
<td>100</td>
<td>141</td>
<td>7</td>
<td>7.0</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
<td>5</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Institute for Public Health Nis
Our study in Institute of Public Health shows that CDAD was diagnosed in four (4%) from 100 involved patients.

This finding agrees with previous report of Berg RJ. Kuijper EJ. Claas ECJ. Rapid diagnosis of toxinogenic Clostridium difficile in faecal samples with internally controlled real-time PCR. Clinical Microbiology and Infectious Diseases 2006. 12: 178-96.

who detected toxins of *C. difficile* in stool specimens in 6 (7.05 %) of 85 hospitalized patients.
The find of followed clinical parameters in patients with presence C. difficile in stool samples

Institute for Public Health Nis

<table>
<thead>
<tr>
<th>Group</th>
<th>Serial number of isolates</th>
<th>Presence of toxin in stool samples</th>
<th>Number of evacuee stool /24h</th>
<th>Number of leucocytes / µL</th>
<th>Body temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td>1</td>
<td>Da (yes)</td>
<td>7</td>
<td>11450</td>
<td>38.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Da (yes)</td>
<td>8</td>
<td>12350</td>
<td>38.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Da (yes)</td>
<td>7</td>
<td>12500</td>
<td>38.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Da (yes)</td>
<td>10</td>
<td>18000</td>
<td>39.7</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Ne (no)</td>
<td>5</td>
<td>9850</td>
<td>37.8</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Ne (no)</td>
<td>6</td>
<td>7650</td>
<td>37.9</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Ne (no)</td>
<td>3</td>
<td>9450</td>
<td>37.6</td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>Ne (no)</td>
<td>2</td>
<td>8745</td>
<td>37.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Ne (no)</td>
<td>1</td>
<td>7650</td>
<td>36.9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Ne (no)</td>
<td>3</td>
<td>6750</td>
<td>37.0</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Ne (no)</td>
<td>2</td>
<td>8950</td>
<td>36.7</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Ne (no)</td>
<td>1</td>
<td>5950</td>
<td>37.2</td>
</tr>
</tbody>
</table>
### Statistical comparison of followed parameters of patients with diarrhoea caused C. difficile and patients control group with find C. difficile

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with diarrhoea caused C. difficile</th>
<th>Patients control group with find C. difficile</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body temperature (°C)</td>
<td>38.55 ± 0.785</td>
<td>36.98 ± 0.192</td>
<td>0.01</td>
</tr>
<tr>
<td>Number of evacuees stool /24h</td>
<td>7.75 ± 0.96</td>
<td>1.8 ± 0.84</td>
<td>0.016</td>
</tr>
<tr>
<td>Number of leucocytes / µL</td>
<td>13575 ± 2986.22</td>
<td>7609 ± 1282.64</td>
<td>0.016</td>
</tr>
<tr>
<td>Duration of antibiotics therapy who previous CDAD (in day; day = 24h)</td>
<td>15 ± 1.41</td>
<td>1 ± 2.24</td>
<td>0.016</td>
</tr>
<tr>
<td>Duration of stay in hospital before appearance of diarrhoea (in day; day = 24h)</td>
<td>17.5 ± 2.52</td>
<td>7.4 ± 3.05</td>
<td>0.016</td>
</tr>
<tr>
<td>Age (expressed in years)</td>
<td>57 ± 12.57</td>
<td>55.2 ± 13.88</td>
<td>0.905</td>
</tr>
</tbody>
</table>

Institute for Public Health Nis
Our results from the study performed in Institute for Public Health Nis are in correlation with so far reported studies in the mean that all patients with positive toxins of *C. difficile* in stool specimens were underwent antibiotic treatment longer than fourteen days.
Recommendations for Hospitals

- Hospitals should conduct surveillance for CDAD
  - Recently proposed surveillance recommendations\(^1\)
- Early diagnosis and treatment important for reducing severe outcomes and should be emphasized
  - Subset of epidemic isolates tested: metronidazole susceptible
- Strict infection control: CDC Fact Sheet\(^2\)
  - Contact precautions for CDAD patients
  - An environmental cleaning and disinfection strategy
  - Hand-washing with CDAD patients in outbreak
- Further research needed
  - Role for antimicrobial controls in stemming this epidemic

\(^{1}\)McDonald et al. *Infect Control Hosp Epidemiol* 2007; 28:140-145
\(^{2}\)See CDC *C. difficile* Fact Sheets: [http://www.cdc.gov/ncidod/dhqp/](http://www.cdc.gov/ncidod/dhqp/)
ESBL and MBLs
1359 isolates from the family of *Enterobacteriaceae* of different clinical materials of the hospitalised patients during 2007.
Presence of *Enterobacteriaceae* resistant to beta lactam antibiotics in 2002. given in percentage
Presence of *Enterobacteriaceae* resistant to beta lactam antibiotics in 2002. given in percentage.
Presence of *Enterobacteriaceae* resistant to beta lactam antibiotics in 2007. given in percentage
Presence of *Enterobacteriaceae* resistant to ampicillin in 2002 and 2007 given in percentage.
Presence of *Enterobacteriaceae* resistant to ceftriaxone in 2002 and 2007, given in percentage
Presence of *Enterobacteriaceae* resistant to aminoglycoside, ciprofloxacin and carbapenem in 2002 given in percentage
Presence of *Enterobacteriaceae* resistant to aminoglycoside, ciprofloxacin and carbapenem in 2007. given in percentage
Presence of *Enterobacteriaceae* resistant to ciprofloxacin in 2002 and 2007, given in percentage.
Presence of ESBL+ isolates with *Enterobacteriacea* resistant to aminoglicozide, ciprofloxacin and carbapenem in 2007. given in percentage
Presence of ESBL+ isolates with *Enterobacteriaceae* and nonfermentive Gram negative bacilli
Presence of ESBL+ isolates with *Enterobacteriaceae*
Pseudomonas aeruginosa
Gr- bacilli-neferm.
Presence of ESBL+ isolates with Enterobacteriaceae and nonfermentive Gr-bacilli

Presence of ESBL+ isolates with Enterobacteriaceae and nonfermentive Gr-bacilli
We believe that it is now mandatory for scientists and clinicians to come together to discuss the recent situation and its possible solutions.
Clinical evidence suggests that early use of appropriate empiric antibiotic therapy improves patient outcomes in terms of:

- reduced mortality
- reduced morbidity
- reduced duration of hospital stay
EFFICIENT THERAPY
prolongs the time interval of the use of new classes of antimicrobial drugs