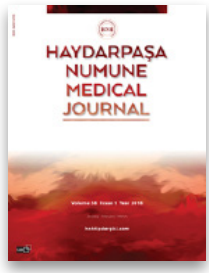
HAYDARPAŞA NUMUNE MEDICAL JOURNAL

DOI: 10.14744/hnhj.2019.43650
Haydarpasa Numune Med J 2021;61(1):71–76

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ORIGINAL ARTICLE

# Cumulative Antibiogram Test Results of Isolated Microorganisms from Blood Culture Samples at Haydarpaşa Numune Training and Research Hospital

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## Abstract

**Introduction:** In this study, we aimed to investigate cumulative antibiogram results of microorganisms isolated from bloodstream infections of patients admitted to our hospital to provide a guideline for the empirical treatment.

**Methods:** The blood culture samples sent to our laboratory between January 2017 and September 2018 were incubated in BACT-ALERT 3D (bioMerieux- France) system. The microorganisms were identified with conventional methods and MALDI-TOF MS (bioMerieux- France), and the antibiotic susceptibility test was performed with VITEK-2 (bioMerieux- France) automated systems. The cumulative antibiogram data were analyzed according to Clinical and Laboratory Standards Institute M39-A4 criteria. Data analysed for our study were retrieved from our hospital information system. The cumulative antibiotic sensitivity limit for empirical treatment was considered to be at a level >90%.

**Results:** In this study, 969 isolates were analysed. Vancomycin, teicoplanin, linezolid, and tigecycline were effective in *Enterococcus* species. In addition to these antibiotics, daptomycin was evaluated to be effective against *Staphylococcus aureus*. Carbapenems and tigecycline were effective in *Escherichia coli* isolates, and they can be used as empiric antibiotics but not against *Klebsiella pneumoniae* isolates. Amikacin was effective against other bacteria in Enterobacteriaceae members. However, no appropriate antibiotic was detected to be effective for the empirical treatment of non-fermentative Gram-negative rods, including *Pseudomonas aeruginosa* and *Acinetobacter baumannii* infections. *Candida albicans* was sensitive to all antifungal agents. Amphotericin B, micafungin and caspofungin were appropriate for empirical treatment of other Candida species.

**Discussion and Conclusion:** Empirical treatment options for Gram-negative rods are highly limited and we believe that training programmes scheduled by the infection control committee and close monitoring of the associated processes will improve and decrease antibiotic resistance rates.

**Keywords:** Cumulative antibiogram; microorganism; susceptibility rate.

It takes at least 2-3 days to evaluate and report patient samples sent to the Medical Microbiology laboratory due to suspected infection. It is vital to initiate appropriate empirical therapy to prevent the progression of the infection during this time. Nowadays, there is an increasing problem of multiple resistance/pan-resistant against antimicrobials worldwide. A cumulative report of the antimicrobial susceptibility data is important to guide the clinician in the selection of empirical antimicrobial therapy, as well as for monitoring changes in sensitivity rates, establishing sur-

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**Submitted Date (Başvuru Tarihi):** 04.12.2018 **Accepted Date (Kabul Tarihi):** 16.03.2019

veillance programs and for the guidance of the antimicrobial usage policies. Thus, Clinical and Laboratory Standards Institute (CLSI) has published a cumulative antibiogram preparation guideline[1]. In this guideline, the points to be considered when preparing the cumulative antibiogram report are stated. According to the guideline, a cumulative antibiogram report is prepared by examining the sensitivity rates of microorganisms isolated in a certain time (usually one year) against antimicrobials. In our study, we evaluated the cumulative susceptibility results of microorganisms grown in blood cultures. With this evaluation, we aim to provide guiding data to clinicians for the selection of empirical treatment in bloodstream stream infections in our hospital.

## Materials and Methods

Blood culture samples sent from various services, including Anesthesia and Reanimation service, were incubated in the automated system (BACT-ALERT 3D, bioMerieux-France) for five days between January 2017 and September 2018. During this period, samples that gave positive signals were taken from the device and Gram-stained and inoculated on chocolate agar, sheep blood agar and MacConkey agar (bioMerieux- France). Gram staining result was reported to the relevant clinic as a preliminary report. Colonies growing at the end of the overnight incubation were identified using conventional methods and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS, bioMerieux-France) device. VITEK-2 (bioMerieux-France) automated system was used for antibiotic susceptibility tests. When necessary, gradient test and disk diffusion methods were also used to confirm antibiotic susceptibility test results in the automation system. Data analysed for our study were retrieved from our hospital information system. Susceptibility results were interpreted according to expert guidelines of the European Committee for Antimicrobial Susceptibility Tests (EUCAST) [2]. Routinely tested antibiotics were included in the preparation of the reports and the sensitivity rates of these antibiotics were reported.

In our study, the CLSI M39-A4[1] guideline was considered to analyze cumulative antibiogram sensitivity data; the cumulative antibiotic sensitivity limit for empirical treatment was considered to be at a level of >90%[3].

When evaluating antimicrobial susceptibility rates, the number (>=30) of isolated microorganisms of the same species/genus were included in this study. If the same type of microorganism reproduced more than once from a patient within the time evaluated, only the first isolate was included in this study. Sensitivity percentages were calculated by taking the number of isolates tested.

According to the CLSI recommendation, *Enterococcus faecalis* (*E. faecalis*), *Enterococcus* faecium (*E. faecium*), *Enterococcus* spp. (include *E.faecalis* and *E.faecium*), methicillin-sensitive and resistant *Staphylococcus aureus* (*S. aureus*) were analyzed separately and in total and sensitivity rates were presented.

The data of Enterobacteriaceae and nonfermentative Gram-negative rods are presented in two separate tables. Since our study was conducted retrospectively by evaluating patient results, Ethics Committee approval was not obtained.

## Results

In this study, 969 microorganisms were analyzed. The antimicrobial susceptibility rates of evaluated isolates are presented in Tables 1, 2, 3 and 4 for Gram-positive bacteria, Enterobacteriaceae, nonfermentative Gram-negative rods and *Candida spp*., respectively. The antibiotic groupings presented in the tables were made according to the limited reporting recommendations of the Turkish Microbiology Society's Standardization of Antibiotic Susceptibility Tests (ADTS) Working Group[4].

## Discussion

In the light of previous data, planning of empirical treatment is an important step in controlling the infection in the initial period, preventing complications, improving patient prognosis and reducing costs. Cumulative antibiogram reports should be published at regular intervals in a standard way and sensitivity rates should be compared due to previous reports. Since cumulative antibiogram data were prepared for the first time in our hospital, they could not be compared with the data of previous years. In our study, it was planned to prepare a report for the critical care service, but because the number of some microorganisms was insufficient in terms of statistical evaluation, blood culture data sent from other services were also included in this study. In this respect, *Enterococcus spp*., *Staphylococcus aureus* (*S. aureus*) from Gram-positive cocci, Enterobacteriaceae family from Gram-negative bacteria, *Acinetobacter baumannii* (*A. baumannii*) and *Pseudomonas aeruginosa* (*P.aeruginosa*) from nonfermentative Gram-negative rods, and *Candida spp*. were analysed due to isolating the number of >30.

While the sensitivity of *E. faecalis* detected in our blood culture isolates against ampicillin was 93.7%, this rate was

**Table 1.** Distribution of antibiotic sensitivity percentages for Gram-positive cocci according to selective reporting groups

| | Susceptibility % | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Grup A | | | | Grup B | | | | | Grup C | | |
| Microorganisms (no.)* | AMP (n) | | | | VA (n) | TEC (n) | | LZD (n) | HLG. (n) | TIG (n) | | |
| *E. faecalis* (69) | 93.7 (63) | | | | 100 (68) | 100 (67) | | 100 (68) | 52.6 (57) | 100 (50) | | |
| *E. faecium* (42) | 0.0 | | | | 90.5 | 90.5 | | 100 | 56.4 (39) | 100 (32) | | |
| *Enterococcus spp.* (118**) | 55.4 (112) | | | | 96.6 (117) | 96.6 (117) | | 100 | 54.4 (103) | 100 (89) | | |
| | Grup A | | | | Grup B | | | | | Grup C | | |
| | P (n) | OX (n) | E (n) | CLIN (n) | VA (n) | TEC (n) | CIP (n) | LEV (n) | GEN (n) | LZD (n) | TIG (n) | DAP (n) |
| (MSSA) (98) | 20.4 | S | 90.6 (96) | 90.5 (95) | 100 (97) | 100 (96) | 91.8 | 95.7 (93) | 97.4 (78) | 100 | 100 (68) | 100 (65) |
| (MRSA) (38) | | R | 65.8 | 78.9 | 100 | 100 (36) | 60.5 (38) | 59.5 (37) | 75 (36) | 100 (37) | 100 (36) | 100 (34) |
| *S. aureus* (136) | 17.2 | 72.1 | 82.8 | 87.2 | 100 (135) | 100 (132) | 85.3 | 85.4 (130) | 90.4 (114) | 100 (135) | 100 (95) | 100 (102) |

*It is arranged according to the CLSI M39-A4 guideline (if the number of isolates studied is different from the total number of isolates in the antibiotic susceptibility test, the sensitivity percentage should be written first, and the number of isolates should be specified in parentheses).

**Include other seven strains of Enterococcus spp. were non-*E.faecium* and non-E.faecalis.

AMP: Ampicillin; VA: Vancomycin; TEC: Teicoplanin; LZD: Linezolid; DAP: Daptomycin; HLG: High level gentamycin; TIG: Tigecycline; P: Penicillin; OX: Oxacillin; E: Erithromycin; CLIN: Clindamycin; CIP: Ciprofloxacin; LEV: Levofloxacin.

**Table 2.** Distribution of sensitivity percentages for Enterobacteriaceae according to selective reporting groups

| | Susceptibility % | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Grup A | | Grup B | | | | | | | Grup C | | |
| Microorganisms (n)* | AMP (n) | GEN (n) | AMC (n) | TZP (n) | CAZ (n) | AK (n) | CRO (n) | CIP (n) | IMP | MEM (n) | ERT (n) | TİG (n) |
| *Escherichia coli* (201) | 22.7 (172) | 68.1 (163) | 30.6 (98) | 71.6 | 37.8 | 72.6 | 40.1 (177) | 35.3 | 98.0 (50) | 97.5 (198) | 95.5 (179) | 99.5 (191) |
| *Klebsiella pneumoniae* (198) | 0 (51) | 50.4 (113) | 25.5 (51) | 33.3 (135) | 23.3 (127) | 53.3 (135) | 26.0 (127) | 34.8 (132) | | 63.6 (184) | 51.2 (123) | 52.3 (128) |
| Other *Enterobacteriaceae* (84) | 13.6 (59) | 69.9 (83) | 32.2 (59) | 70.3 (74) | 55.4 (83) | 90.4 (83) | 42.0 (69) | 61.5 (83) | | 81.5 (81) | 83.1 (59) | 47.0 (83) |

*It is arranged according to the CLSI M39-A4 guideline (if the number of isolates studied is different from the total number of isolates in the antibiotic susceptibility test, the sensitivity percentage should be written first, and the number of isolates should be specified in parentheses).

AMP: Ampicillin; GEN: Gentamycin; AMC: Amoxicillin and Clavulanic Acid; TZP: Piperacillin+Tazobactam; CAZ: Ceftazidime; AK: Amikacin; CRO: Ceftriaxone; CIP: Ciprofloxacin; IMP: Imipenem; MEM: Meropenem; ERT: Ertapenem; TIG: Tigecycline.

55.4% for all *Enterococcus spp*. Vancomycin, teicoplanin, linezolid and tigecycline were found suitable for empirical treatment against *Enterococcus spp*. bacteria, ampicillin is seen as an empirical treatment option for E. faecalis in addition to these antibiotics. Resistance to vancomycin was detected only in four *E. faecium* (9.5%), and the resistance rate was 3.4% in total *Enterococcus spp*. In studies conducted on blood culture samples in our country, Er et al.[5] reported

**Table 3.** Distribution of susceptibility percentages for nonfermentative Gram-negative bacteria according to selective reporting groups

| | Susceptibility | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Grup A | | | Grup B | | | | Grup C | |
| **Microorganisms (n)*** | **CAZ (n)** | **TZP (n)** | **Gen (n)** | **AK (n)** | **IMP (n)** | **MEM (n)** | **CIP (n)** | **TOB(n)** | **LEV(n)** |
| *Pseudomonas aeruginosa* (80) | 61.8 (55) | 50.0 (54) | 75.6 (41) | 70.9 (55) | 70.9 (54) | 70.4 (54) | 74.5 (55) | 80.8 (52) | 72.9 (48) |
| | Grup A | | | | | Grup B | | Grup C | |
| | **CAZ (n)** | **IMP (n)** | **MEM (n)** | **Gen (n)** | **AK (n)** | **CIP (n)** | **TZP (n)** | **[1]TOB (n)** | **[1]LEV (n)** |
| *Acinetobacter baumannii complex* (80) | 8.9 (45) | 5.4(74) | 7.5 | 45.9 (61) | 37.5 | 7.5 | 5.6 (54) | 54.2 (59) | 5.7 (70) |

* It is arranged according to the CLSI M39-A4 guideline (if the number of isolates studied is different from the total number of isolates in the antibiotic susceptibility test, the sensitivity percentage should be written first, and the number of isolates should be specified in parentheses).

[1]The TOB and LEV sensitivity percentages according to EUCAST.

CAZ: Ceftazidime; TZP: Piperacillin+Tazobactam; GEN: Gentamycin; AK: Amikacin; IMP: Imipenem; MEM: Meropenem; FEP: Cefepim; CIP: Ciprofloxacin; TOB: Tobramycin; LEV: Levofloxacin.

**Table 4.** Sensitivity percentage for *Candida spp.*

| | % Sensivity | | | | |
|---|---|---|---|---|---|
| **Number of organism (n)*** | **Amphotericin B** | **Fluconazole** | **Caspofungin** | **Miconazole** | **Voriconazole** |
| *Candida albicans* (46) | 100 (43) | 100) | 100 | 100 | 100 |
| [1]*Candida spp.* (51) | 100 (44) | 86.1 (43) | 87.5 (48) | 98.0 (49) | 100 (42) |

[1]*C. parapsilosis* (17), *C. tropicalis* (16), *C. glabrata* (6), *C. kefyr* (4), *C. krusei* (4), *C. lusitaniae* (2), *C. dubliniensis* (1), *C. haemuloni* (1).

4.3% vancomycin resistance for *E. faecium*. In the study conducted by Gülfem[6] in 2013, vancomycin resistance was not reported in their study. Iraz et al.[7] reported vancomycin resistance at a rate of 4% and 23% for *E. faecalis* and *E. faecium*; they isolated from various clinical specimens, respectively. In a study conducted in intensive care units in the south of India[8], this rate was reported as 11.9% in the blood and other samples. According to a report from Europe and the United States of America (USA), VRE isolated from invasive infections increased from 4.7% to 20.3% in Europe, while in the USA It was reported that increasing resistance rates until 2010 (from 60% to 80.7%) and decreased relatively after 2011 (from 75.7% to 68.4%)[9].

The rate of methicillin-resistant *Staphylococcus aureus* (MRSA) among the *S.aureus* isolates was found at 27.9%. Er et al.[5] found the rate of MRSA 71.7%, Nazik et al.[10] 48.1%, and Çetinkol et al.[11] 25.6% in *S.aureus* isolates from blood culture samples in our country. Öksüz et al.[12] found resistance to tigecycline at a rate of 2% in MRSA strains isolated from various clinical samples, and they did not report resistance to linezolid, daptomycin, and vancomycin. All *S.aureus* isolates in our study were 100% sensitive and suitable for empirical treatment for vancomycin, teicoplanin, linezolid, tigecycline and daptomycin. In addition to these antibiotics, ciprofloxacin, levofloxacin and gentamycin can be used for empirical therapy in MSSA isolates. In a study conducted in the USA, in a group of pediatric patients with blood culture samples, the rate of MRSA was reported to be 43%[13].

In *Escherichia coli* (*E. coli*), imipenem, meropenem, ertapenem and tigecycline are seen as suitable options for empirical treatment. According to the susceptibility results of *Klebsiella pneumonia* (*K. pneumoniae*) isolates, there is no empirical treatment option. Among other Enterobacteriaceae members other than *Escherichia coli* and *K. pneumoniae* species, amikacin was the only option most suitable for empirical treatment. Since the number of tests for imipenem, one of the antibiotics in the carbapenem group, was low, the results could be reflected only for *E.coli* in table 2. The susceptibility test of imipenem was performed on 21 strains of *K. pneumoniae* isolates and 24 strains of other Enterobacteriaceae members, and the sensitivity rates were

52.4% and 62.5%, respectively. In a study conducted with blood culture samples in our country, *E. coli* isolates were resistance at a rate of 4.7% (95.3% sensitive) for imipenem and meropenem and 31.5% (68.5% sensitive) for imipenem and meropenem in *K. pneumoniae* isolates[14]. In a review study evaluating the resistance rates of Gram-negative bacteria in intensive care units for device-related infections in the USA[15], carbapenem resistance rates were 3.6%-4.6%, 11.2%- 12.8% and 1.9% -3.5% for *Enterobacter spp.*, *K. pneumoniae/oxytoca* and *E. coli*, respectively. In our study, sensitivity rates for meropenem and ertapenem in *E. coli*, *K. pneumoniae* and other Enterobacteriaceae members were 97.5%, 95.5%, 63.6% and 51.2%, 81.5% and 83.1%, respectively. In the study from the USA[15], higher sensitivity was found in intensive care units compared to our study, but similar to our results, *K. pneumoniae* isolates were more resistant to carbapenems than *E. coli*[15].

In our study, among the nonfermentative Gram-negative bacteria, *Acinetobacter baumannii* (*A. baumanni*i) and *Pseudomonas aeruginos*a (*P. aeruginosa*), antibiotic susceptibility rate was not suitable for empirical therapy. In a study from our country between 2012 and 2013, isolates from various clinical specimens were evaluated according to EUCAST criteria, imipenem and meropenem susceptibilities were reported as 13.7% and 12.6% in *A. baumannii* isolates and 69.7% and 66.9% in *P. aeruginosa* isolates, respectively[16]. Barış et al.[17] reported 5.7% and 65% imipenem sensitivity in *A. baumannii* and *P. aeruginosa* strains isolated from different clinical samples, respectively. In our study, the sensitivity rate to imipenem and meropenem was found 5.4% and 7.5% in *A. baumanni* and 70.9% and 70.4% in *P. aeruginosa,* respectively. Our sensitivity rates for *A. baumannii* and *P. aeruginosa* are similar to two other studies conducted in our region.

The high resistance rates detected in Enterobacteriaceae and nonfermentative Gram-negative bacteria and the problems experienced in treatment have brought the use of polymyxin group antibiotics back to the agenda in recent years. In recent studies, it has been reported that neither automated system nor gradient tests are suitable for detecting colistin sensitivity; it was suggested by EUCAST and CLSI that the colistin sensitivity test should be studied with the broth microdilution method[2,18]. Since we do not use the broth microdilution method for colistin in our routine laboratory practices, the colistin sensitivity rates of the automated system are not reflected in our cumulative antibiogram results. In this case, it is inevitable to apply a standard and practical broth microdilution method in routine studies for indications where the use of colistin as the only option is mandatory.

The *C. albicans* isolates (n=46) in our study were 100% sensitive to all antifungals tested in accordance with empirical treatment. Non-*albicans Candida* isolates (n=51), were *C. parapsilosis* (n=17), *C. tropicalis* (n=16), *C. glabrata* (n=6), *C. kefyr* (n=4), *C. krusei* (n=4), *C. lusitaniae* (n=2), *C. dubliniensis* (n=1), and *C. haemulonii* (n=1). Sensitivity rates were calculated by considering intrinsic resistance to fluconazole in *C. krusei* isolates. Accordingly, amphotericin B, miconazole and voriconazole are suitable agents for empirical treatment. Although it was recommended to report the MIC result without comment in the antifungal susceptibility test for amphoteric B[19], the MIC value was <1 mg/L in all *Candida spp*., and accordingly, it was reflected as sensitive in the reports. Çalışkan et al.[20] isolated *Candida spp*. from blood culture samples sent from intensive care units between January 2009 and December 2012. The distribution and susceptibility rates to four antifungals were examined. Accordingly, two of the 33 *C. albicans* strains were less susceptible to amphotericin B and the other 31 strains were 100% susceptible to voriconazole, flucytosine, fluconazole. The sensitivity rates of other *Candida spp*. isolated in the same study are similar to the rates of our study.

In a similar study conducted by Özkaya et al.[21], 94.1% of 17 *C. albicans* strains against amphotericin B, 74.2% of 31 *C. parapsilosis* strains against voriconazole, 93.5% against flucytosine and amphotericin B, and 100% of all *Candida spp*. were found susceptible against caspofungin.

As a result, regular analysis of cumulative antibiogram data on hospital basis and updating antibiotic use policies according to these data will provide meaningful information to clinicians for empirical treatment options and contribute to reducing resistance rates.

**Ethics Committee Approval:** Retrospective study.

**Peer-review:** Externally peer-reviewed.

**Authorship Contributions:** Concept: R.A., N.K.; Design R.A., N.K., S.A.; Data Collection or Processing: R.A., M.A.; Analysis or Interpretation: R.A., N.K.,M.A., S.A.; Literature Search: R.A., N.K.; Writing: R.A., N.K., M.A., S.A.

**Conflict of Interest:** None declared.

**Financial Disclosure:** The authors declared that this study received no financial support.

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