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Sensitivity of Antibiotics Containing Citrobacter Bacteria

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Abstract

Enterobacteria are one of the most common infectious agents among opportunistic pathogens. Both among community-acquired and hospital-acquired infections, such as intestinal and extra-intestinal localization (urinary, respiratory tract infections, intra-abdominal infections, skin and soft tissues, as well as generalized infections), the cases with resistant citrobacteria are quite common to observe. Recently, Citrobacter bacteria are getting widely spread as determinants of antibiotic resistance through its representatives. This fact greatly complicates the therapy towards infections.

Keywords: citrobacter, resistance, antibiotics, disco-diffuse method, tetracyclines, aminoglycosides, quinolones, beta-lactams, chloramphenicol.

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1. Introduction

Citrobacter bacteria are widely distributed in the environment, they can be found in water drains, soil and various foods; can be isolated from the urinary tract and intestines of a human, cattle, horses, birds, rodents, dogs, reptiles, insects [4, 5].

Citrobacter is often received due to some medical devices and other facilities that are used in medical institutions, this circumstance causes occurrence of hospital infections for a person with signs of opportunistic pneumonia, bacteremia, wound infections, and lesions of the urinary tract [1].

Citrobacter bacteria in human being organism are more often found when poisoned with food, diagnosed gastroenterocolitis, urinary and biliary tract infections, bacteraemias, endocarditis, osteomyelitis, otitis, meningitis, purulent complications caused by surgery, organ abscesses [6, 4].

It is known that more often the lesions are developing after horizontal transmission of Citrobacter, through living together-contacts and through the fecal-oral way. There is also evidence about vertical transmission cases, from mother to embryo [2, 7]. Citrobacter have a high resistance to antibiotics [9, 8].

So, on the one hand, we have wide spreading of Citrobacter bacteria and its antibiotic resistance, and on the other hand, the contradiction between the need to study its sensitivity to antibiotics and not sufficient research base towards the topic in theory and practice determines the importance of the issue offered to discuss.

The research data are relevant and of interest for ordinary people as well as for specialists in the field of medicine and biology.

2. Methods and Materials

As the material for this research we took 10 strains Citrobacter, isolated from the patients with acute intestinal infections. For the bacteriological examination we used the nutrient medium:

1. 0.3 %, 0.7 %, 1.5 %; medium Endo (State Research Center for Applied Microbiology, Obolensk).

2. Bismuth sulfite agar (`Biotechnology', Moscow).

3. Meat peptone agar (Scientific institution `Nutrient Medium', Makhachkala).

4. Meat peptone broth (Scientific institution `Nutrient media', Makhachkala).

The combination of morphological, tinctorial, cultural and biochemical properties allowed identifying the cultures we have identified as Citrobacter freundii.

Microorganisms' sensitivity was determined to 10 antimicrobial agents via the disco-diffusion method in accordance with [MYK] 4.2.1890-04 -- methodology guide [3].

The disco-diffusion method is based on antibiotic ability to diffuse from paper disks, impregnated with them, into the medium/environment and inhibit microorganisms' growth that were seeded on the agar surface.

To determine the sensitivity through the disk-diffusion method, nutrient mediums used are the same as used within the agar dilution method, and, therefore, the same methods on quality control are applied.

When determining the sensitivity via the disk-diffusion method, a standard inoculum is used, which corresponds to 0.5 according to the McFarland standard, containing approximately $1.5x10^8$ CFU/cm^3. Inoculum should be used during 15 minutes since it has been prepared. Two methods can be used to inoculate agar plates.

Immediately afterwards, the Petri plates are placed into a thermostat upside down and incubated at 35 °C for 18--24 hours (the time depends on microorganism type to be tested). The expand within the time interval between the disks contacting with the medium and the incubation beginning that, consequently, is equal to the starting point

in the culture growth, leads both to `pre-diffusion of antibiotic' into the agar and to the increase of the diameter in growth suppression zone. After incubation, the plates are placed upside down on a dark matte surface so that the light falls on them at the angle of 45° (in reflected light). The diameter related to the growth inhibition zones is measured with the accuracy of 1 mm (preferably to use a caliper).

At measuring the growth inhibition zones, the process is targeted at the zone demonstrating a complete suppression in visible growth. One should not pay attention to very small colonies detected within the growth inhibition zone if there are some special lighting conditions and if there is a barely noticeable deposit at the edge of the zone.

Large colonies within a clear zone of the growth inhibition indicate about extraneous micro-flora or hetero-resistance among the microorganism population and in this case the repeated microorganism identification is needed with the aim to check what forms the colony and determines the sensitivity of this strain.

To test the sensitivity to antibiotics of the bacteria to have been isolated, we used the standard disks with agents:

1. Groups of tetracycline (doxycycline).
2. Aminoglycosides (gentamicin, kanamycin, streptomycin, neomycin).
3. Quinolones (moxifloxacin).
4. Beta-lactams (ampicillin, amoxicillin).
5. Levomycetinum.

The set with discs containing antibacterial agents has been collected with the view on what antibiotics are used in treatment purposes in hospitals.

3. Results

Outcome analysis are introduced in figures 1--9.

At this testing (Fig. 1) we showed up that 90 % of isolators turned out to be sensitive and only 10 % are low-sensitive.

At this testing (Fig. 2) we showed up that 80 % of isolators turned out to be sensitive and 20 % are low-sensitive.

At this testing `Citrobacter-kanamycin' (Fig. 3) we showed up that 40 % of isolators turned out to be sensitive, 50 % are low-sensitive and 10 % are non-sensitive.

At this testing `Citrobacter-streptomycin' (Fig. 4) we showed up that 50 % of isolators turned out to be sensitive, 40 % are low-sensitive and 10 % are non-sensitive.

At this testing `Citrobacter-neomycin' (Fig. 5) we showed up that 80 % of isolators turned out to be sensitive, 20 % are low-sensitive.

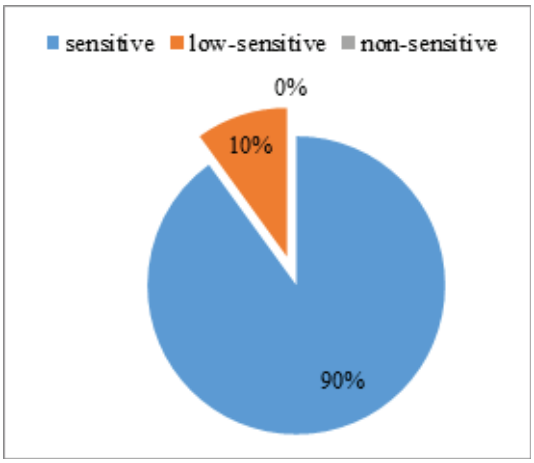

Figure 1: Citrobacter sensitivity to doxycycline.

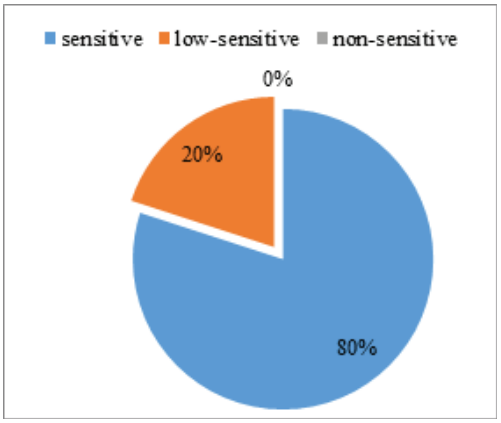

Figure 2: Citrobacter sensitivity to gentamicin.

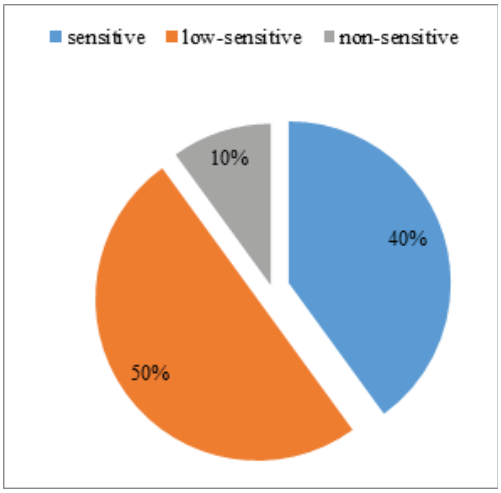

Figure 3: Citrobacter sensitivity to kanamycin.

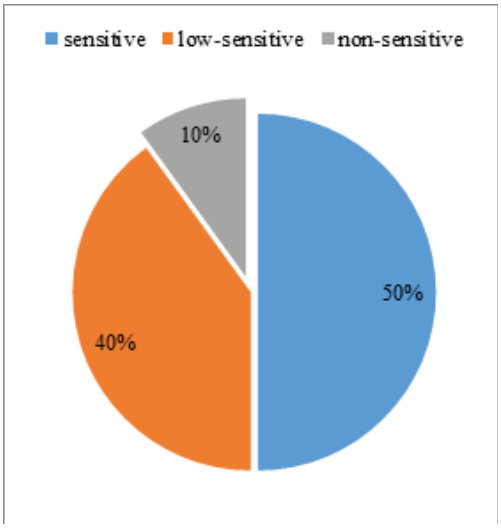

Figure 4: Citrobacter sensitivity to streptomycin.

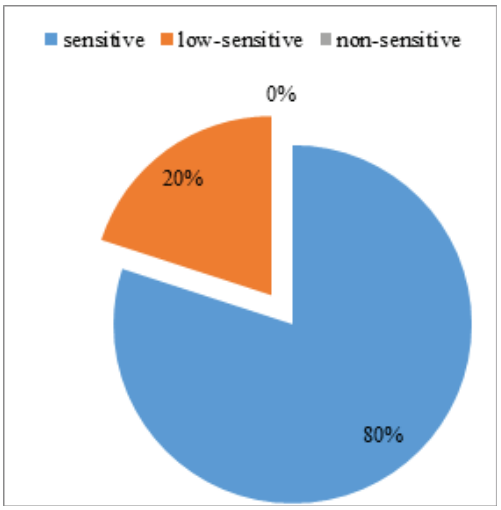

Figure 5: Citrobacter sensitivity to neomycin.

At this testing `Citrobacter-moxifloxacin' (Fig. 6) we showed up that 90 % of isolators turned out to be sensitive, 10 % are low-sensitive.

At this testing `Citrobacter-ampicillin' (Fig. 7) we showed up that 90% of isolators turned out to be sensitive, 10 % are low-sensitive.

At this testing `Citrobacter-amoxicillin' (Fig. 8) we showed up that 80 % of isolators turned out to be sensitive, 20 % are low-sensitive.

At this testing `Citrobacter-levomycetinum' (Fig. 9) we showed up that 30% of isolators turned out to be sensitive, 60 % are low-sensitive and 10 % -- non-sensitive.

To illustrate the data, we put it in table 1.

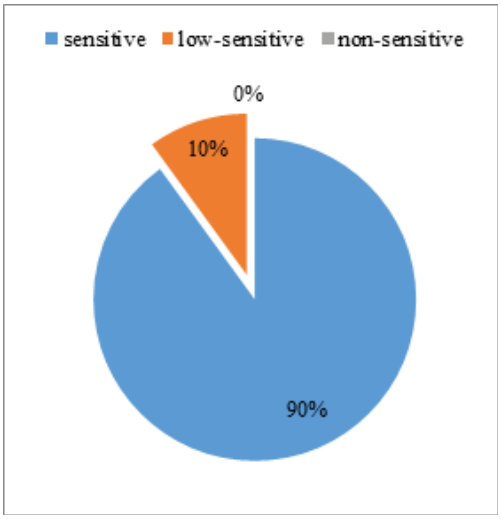

Figure 6: Citrobacter sensitivity to moxifloxacin.

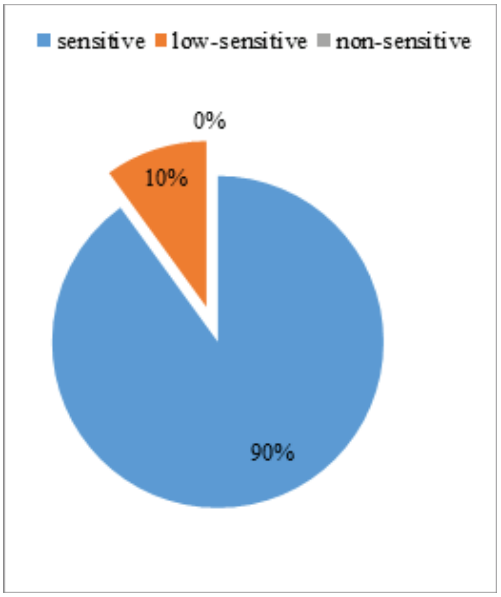

Figure 7: Citrobacter sensitivity to ampicillin.

4. Conclusion

The research proves a wide speread resistancy to a great number of antibacterial agents among Citrobacter isolators segreted at intestinal infections. Due to the empirical study we concluded that Citrobacter bacteria expressed the strong resistance to kanamycin, streptomycin and chloramphenicol. While to doxycycline, gentamicin, neomycin, moxifloxacin, ampicillin, amoxicillin, the majority of isolated citrobacter showed quite good sensitivity.

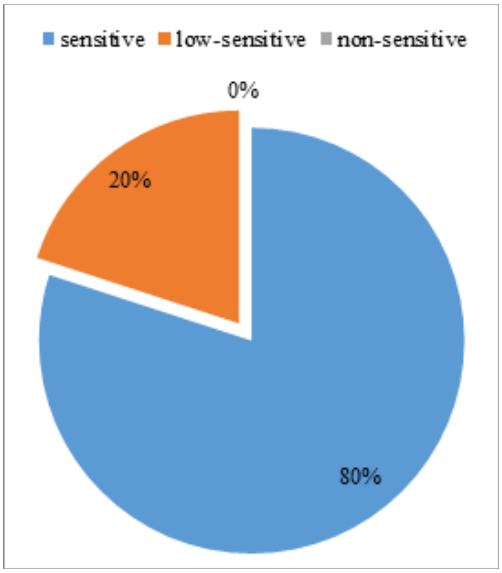

Figure 8: Citrobacter sensitivity to amoxicillin.

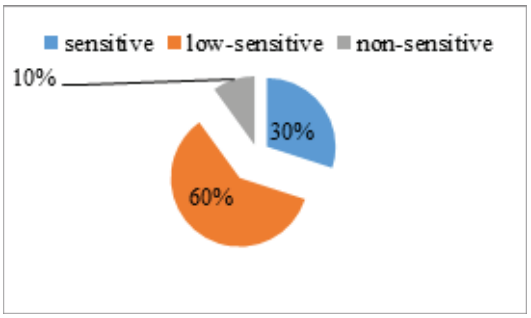

Figure 9: Citrobacter sensitivity to levomycetinum.

TABLE 1: Citrobacter Sensitivity ranking.

Antibiotic	Sensitive	Low-sensitive	Non-sensitive
doxycycline	9	1	0
gentamicin	8	2	0
kanamycin	4	5	1
streptomycin	5	4	1
neomycin	8	2	0
moxifloxacin	9	1	0
ampicillin	9	1	0
amoxicillin	8	2	0
levomycetinum	3	6	1

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