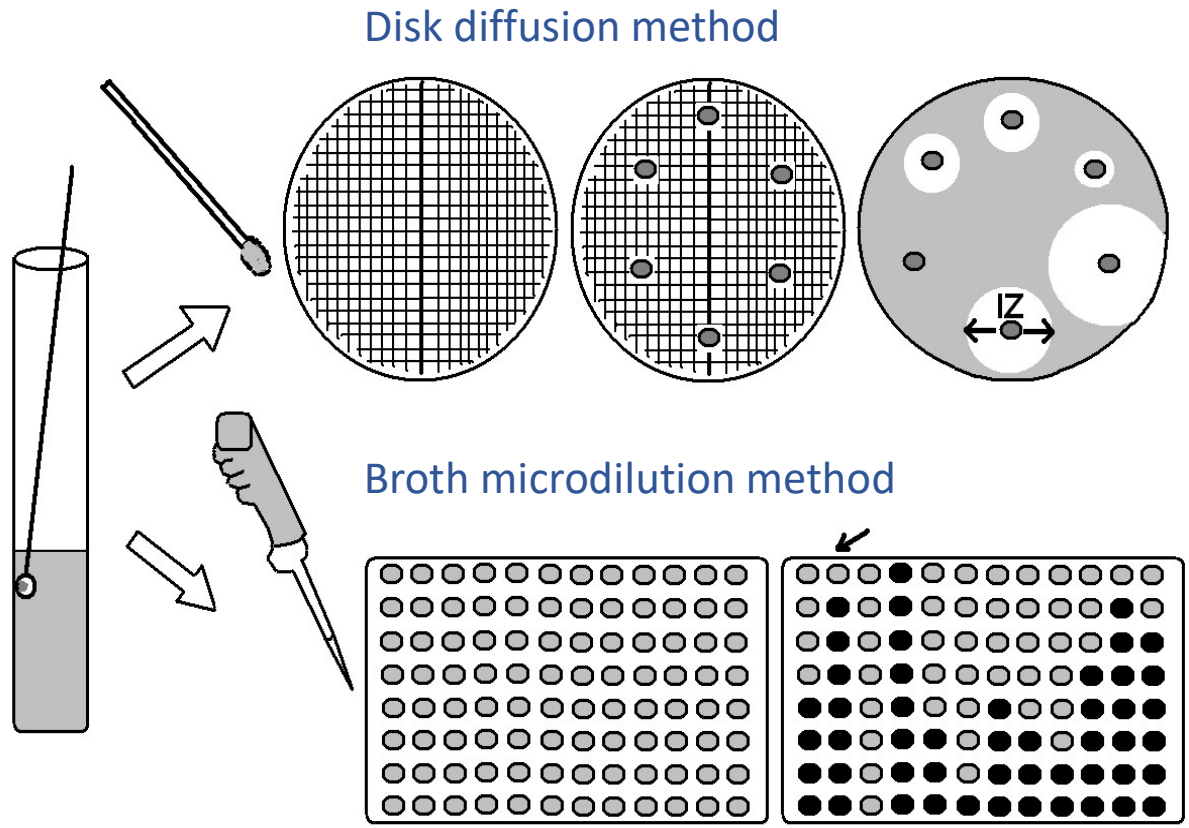
ANTIMICROBIAL SUSCEPTIBILITY TESTING

The main purpose is to test sensitivity (susceptibility) of a given microorganism to known susceptibility/resistence of microorganism to antibiotics and to select the most appropriate drug for treatment.

Qualitative method is also known as disk diffusion method. Antibiotic molecules diffuse out from a disk into the agar creating a concentration gradient of antibiotics. A particular concentration of the antibiotic inhibits bacterial growth (overnight, 37˚C) so an inhibition zone (*IZ*) around the antibiotic disk is detected. Depend on the diameter (mm) of the inhibition zone the result is categorized as *sensitive* or *resistant*. The zones can be read visually or automatically. The results could be influenced by numerous factors (e.g. bacterial inoculum size, thickness of agar) so all the steps should be standardized. Quantitative methods usually performed as broth microdulition method or agar dilution method is valuable especially if determination of minimum inhibitory concentration (*MIC* – the lowest concentration [µg/ml] of antibiotic that inhibits visible growth of a microorganism) is required for treatment of a critical infection. Dependent on pharmacokinetic and pharmacodynamic data of the antibiotic and organ affected, the therapy could be customized.

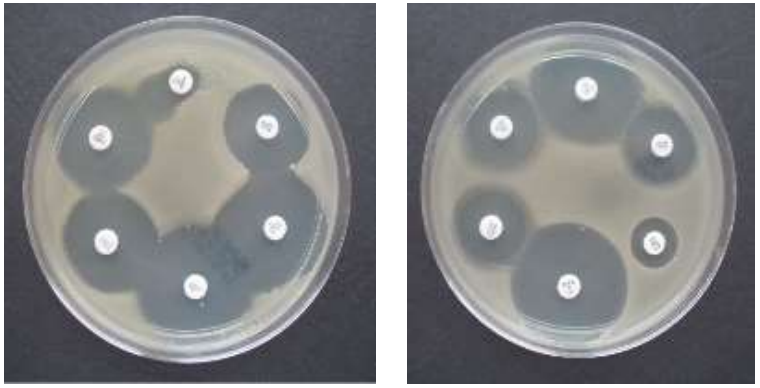


Legend: *Disk diffusion method* – a bacterial inoculum is prepared in nutrient broth (A) and streaked over the agar surface by a cotton swab (B1). Inoculum is dried for 15 minutes and the disks are positioned and cultivated overnight at 37˚C (C1). Size of *IZ*s are read and interpret.
Broth microdilution method – microplate wells are inoculated by a pipette with bacterial inoculum (B2). After overnight cultivation the *MIC* could be read (wells with lowest concentration of antibiotic without visible growth) (C2). The darker wells symbolize growth of culture.

INTERPRETATIONS OF THE RESULTS

Organisms should be categorized as *susceptible* or *resistant* towards a drug dependent on size of the *IZ* on the agar or *MIC* in the microplate wells. The interpretation is based on consensus of global standardization organizations (FDA, CLSI, EUCAST). For example; if the *IZ* for the antibiotic at the position of 6 hours is 25 mm, the zone is larger than the breaking point for the antibiotic (e.g. 22 mm), the bacterial isolate is susceptible to the antibiotic. For example if breaking point for *MIC* of susceptible bacteria is 8 µg/ml and the bacterial culture growth is visible in the first well of second microplate row with concentration of the antibiotic 356 µg/ml, then the bacteria is considered resistant to the antibiotic.

Legend: Disk diffusion method, Left – isolate of *Staphylococcus aureus* susceptible to all antibiotics tested. Middle – IZ of the isolate around co-trimoxazol disk. Right – *Pseudomonas aeruginosa* is naturally resistant to ampicillin (position 9 hours) and co-trimoxazol (position 11 hours). The isolate is susceptible to the rest of antibiotics.

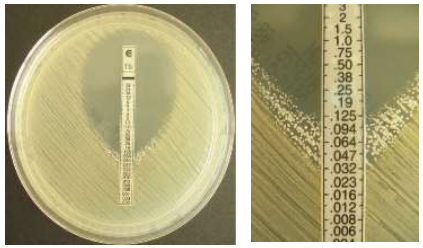


Legend:

Klebsiella pneumoniae isolate susceptible to all antibiotics tested except ampicilin (natural resistance) (left plate, disk at position of 12 hours)

OTHER METHODS; PROCEDURES & INTERPRETATION

Etest (AB Biodisk, Sweden): combination of quantitative and qualitative method. A concentration gradient of an antibiotic on a polymer strip laid on the surface of an inoculated agar plate create an elliptical zone of inhibition. The narrowest end shows the lowest amount of antibiotic needed to inhibit growth i.e. *MIC*.



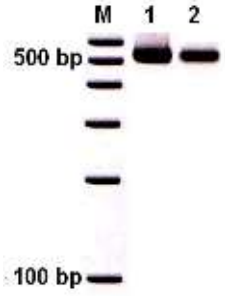
Legend:

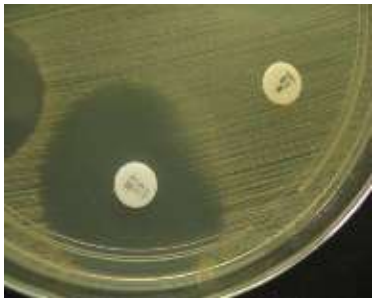
MIC of co-trimoxazol for *S.aureus* isolate is 0,94 µg/ml which is a lower concentration than the breaking point - the isolate is susceptible to the drug.

Automated methods are also available for qualitative and quantitative methods. Most systems used are commercially available panels of antibiotics that contain computerized algorithm for interpretation of the results.

SPECIAL SCREENING OF RESISTANT ORGANISMS

For screening of some key resistance patterns special sets of disks or other methods should be applied.





Legend: the amplicon of *mecA* gene in *S.aureus* isolates from patient 1 and 2 (ref.1)(A), detection of *K. pneumoniae* isolate producing *extended spectrum beta lactamases* (ESBL) - an inhibitor of beta lactamases contained in the central disk show distorsions of the IZ around the betalactams (B), erythromycin disk on the right induce the resistance of clindamycin (chopped IZ on the side of erythromycin)