# CHAPTER 4

## DISCUSSTION

### 1. Comparative study of modified broth disk method and disk diffusion method using BBL disks

The agreements in percentage susceptibility determined by modified broth disk reading at the 3, 6, 8, 18 hrs. intervals and disk diffusion method were demonstrated with Salmonella sp, Shigella sp, E. coli, V. Cholerae and V. parahaemolyticus tested against TC, CM, CO, PN, PM, AM. The agreements were also demonstrated with E. coli and Shigella sp tested against GM and KM respectively. The agreement in the percentage susceptibility derived from the two methods demonstrated the feasibility of utilizing this modified broth disk method for an earlier susceptibility determination of those organisms intended to treat with the above mentioned antibiotics.

Although PM, CL demonstrated some difference in percentage susceptibility to the all the 5 groups of tested organisms tested with the two methods as follow: E. coli showed 43.48% susceptibility against PM as determined by the disk diffusion method; when determined by modified broth disk method reading at 3, 6, 8, 18 hrs. the percentage susceptibility against PM were 95.65%, 95.65%, 95.65%, 95.65% respectively for instance.

However, it was known that PM and CL diffuse very poorly in agar due to the fairly large, strongly cationic molecules which will electrostatically interact with the acidic or sulphate groups in agar medium and consequently the rate of diffusion through the agar is diminished (2, 26, 52). Thus, accuracy of the disk diffusion is less than with other antibiotics, so it is recommended to confirm the results of the disk diffusion with other MIC methods in order to eliminate the ambiguity of the disk diffusion result. These would be taken into further discussion when the modified broth disk results were interpreted in comparison with MIC dilution methods.

As the matter of fact, some possible source of discrepancies in some antibiotic disks must be brought into consideration as: the possible chance of deviation of antibiotic content from the stated potency in disk (23, 48), also the way of handling or keeping the disks to preserve their actual potency and prevent any possible deterioration of the disk (23). Another source of error could be the contamination of disks with bacterial strain of which interfere with the reading of result (23).

The results of modified broth disk method reading at different interval of 3, 6, 8, 18 hrs. showed that the visual end points at 3 hrs. of which were inevitably more subjective than the longer incubation readings. This small variable, of which improved at the 6, 8 hrs. readings for most organism. For example, as reading at 3, 6, 8, 18 hrs. respectively, E. coli demonstrated 26.09%, 21.74%, 21.74%, 17.39% tested against TC and 86.96%, 82.61%, 82.61%, 73.91% tested against CL. This may be because of the very light growth density factor in some tubes which

resulting in a little bit lower percentage of the 3 hrs. reading (32). However, the more frequent discrepancies were demonstrated in those reading at 18 hrs. comparing with the earlier readings. The discrepancies, shown as lower percentage susceptibility than the earlier readings, demonstrated with E. coli tested against CL; V. parahaemolyticus tested against NM, CO, KM; Salmonella sp tested against PM; Shigella sp tested against PM and PN; V. Cholerae tested against KM also detected in AM and GM tested against all 5 groups of organisms.

Some reasons for the variation between the results obtained from 18 hrs. reading and the earlier reading might have been that some antibiotic deteriorate during the 18 hrs. of incubation (55, 34), the reading of the end point should not be extended more than overnight (29). The duration of incubation has also been studied in relationship to the alternations in MIC values. Increasing MIC was noted as incubation was prolonged beyond 12 hrs. (2, 55, 35). The MIC at 3-hrs. reading may be as much as 100-fold lower than the results of overnight tests in some cases (55). Microorganisms with an MIC very close to the concentrations used in the study also tend to appear resistant if the results are not recorded earlier (34) and also may be reported as resistant in one broth disk assay and susceptible in another assay (23). These effects were probably due to progressive inactivation with increasing incubation time. Such effects are more encountered with the relatively unstable penicillins or cephalosporin group (2).

Nevertheless, the present data suggested that the approach adopted to the modified broth disk method with result reading at 3, 6, 8 hrs. showed some agreement with the conventional disk diffusion method at least in some particular organisms to specific antibiotic as previously discussed likely that TC, CM, CO, PN, PM, AM tested against Salmonella sp, Shigella sp, V. parahaemolyticus, E. coli and V. Cholerae. CL tested against Salmonella sp, Shigella sp, V. Cholerae, V. parahaemolyticus. SDZ tested against Salmonella sp, Shigella sp. And GM, KM tested against E. coli and Shigella respectively.

This agreement demonstrated the feasibility of application of the modified broth disk method for a rapid antimicrobial susceptibility determination at least with the above mentioned organisms and antibiotics.

This rapid determination of bacterial susceptibility would decrease significantly the time required to choose the immediate antibacterial therapy especially in urgent infections cases where follow up therapy should referred after this initial therapy.

As the present data showed, the modified broth disk method when applied, the results obtained from reading either at 3, 6 or 8 hrs. should be recommended instead of those from 18 hrs. reading. Comparing the susceptibility results obtained from the 3, 6, 8 hrs. readings it was found to have maximum percentage with closer agreement with disk diffusion method at the 6 hrs. reading. For example the percentage obtained from the 3, 6, 8 hrs. incubation were 71.43%, 71.43%, 69.39% of the susceptibility of Salmonella sp. tested against CM. And 63.27%, 63.27%, 61.22% tested against TC; Also 86.67%, 93.33%, 80% susceptibility of

V. parahaemolyticus tested against NM, 93.33%, 100%, 93.33% susceptibility of V. parahaemolyticus tested against CO. So in this study, the result of reading at 6 hrs. was picked up for further comparison with other MIC dilution method.

Although, the result from reading at 18 hrs. incubation showed some variation from those earlier readings, it should be stressed that the in vitro incubation for susceptibility determination is an artificial situation itself and probably at variance with what happens in vivo. Some antibiotics like penicillin gained progressive deterioration during 18 hrs. of incubation, yet, they may be given to the patient every 4-6 hrs. (55). Similarity, intact host defenses interact with the action of chemotherapeutics on accessible organisms in vivo and cannot be mimicked in vitro.

One possible source of error in the modified broth disk method is the chance of deviation from the stated content of antibiotic in disks (2, 54). Although it is not possible to check the every disks used in test. For quality control of this method, if required, the disks from each cartridge can be checked for appropriate zone sized by the disk diffusion method in order to indicate that the disks are no great problem (54). Another problem was that, it may cause a change in results from sensitive to resistant or vice versa if the MIC of the test organism is very near the break points used in the broth disk method. However, it is possible, if desired, to use more than one concentration in the modified broth disk method by using a different number of disks

in the test and in this way the MIC of organisms can be determined (54).

The modified broth disk procedure appears to be a rapid simple technique for determining the antibiotic susceptibility of enteropathogenics also. The source of antibiotics in readily available disks allows one to perform a one-concentration (or several - concentrations) broth dilution test without the need to prepare and maintain antibiotic solution. Other advantage achieved from this modified broth disk methods included: Earier results obtained. The modified broth disk method is significantly less time consuming than other conventional method. This modification is the ability to determine susceptibility within, say, about 6 hrs. after incubation which decrease the time required to choose the antibacterial choice of therapy. In case of immediate sensitively report required, this modified method can be serve to approximate whether the specific organism sensitive to which antibiotic and at the normal attainable blood level or not by varying the tested concentration. Tube containing antibiotic that inhibit bacterial growth can be subcultured to determine whether this is the bactericidal concentration or not. Easier recorded: In general, the results were easy to read, almost always the tubes were either clear or cloudy enabling a clearcut distinction to be made between the susceptibility or resistant (54). Almost all clinical isolates can be tested for antibacterial susceptibility (23).

## 2. Comparative study of BBL disks and local disks as determined by the modified broth disk method and disk diffusion method

Table 17, 18 showed the agreement of the percentage susceptibility obtained using BBL and local disks in the disk diffusion and modified broth disk method. It was analysed that there were about 33% (13/40) and 50% (80/160) of the number of percentage susceptibility data (Table 16) demonstrated the equal percentage susceptibility obtained using both disks as determined by the disk diffusion and modified broth disk respectively. There were 20% (8/40), 15% (24/160) of data which showed the lower percentage susceptibility obtained using BBL disk than those using local disks as determined by disk diffusion method and modified broth disk method respectively. However, the majority of comparative results demonstrated the higher percentage susceptibility obtained using the BBL disk than those using local disks. The figures of which were 48% (19/40), 35% (56/160) of the total number of data showed higher percentage susceptibility obtained using BBL disks than local disks in the disk diffusion and modified broth disk method respectively.

The percentage susceptibility obtained using different BBL or local disks, when compared as in Table 16, 17, the majority of agreements showed higher or equal percentage susceptibility obtained using BBL than local disks. For example. Testing by the modified broth disk method, about 55% of the percentage sensitivity data demonstrated that the percentage susceptibility obtained using BBL disks were higher than those obtained using local disks and vice versa. Except for the SM and TC disks

of which demonstrated the majority of data showed that BBL disks achieved lower or at least equal percentage susceptibility than those using local disk i.e. 40% and 50% of the data demonstrated the lower and equal percentage susceptibility achieved using the BBL than using the local disks.

Comparing the percentage susceptibility obtained using the two disks and the two method in Table 15. The percentage differences using the two disks were within ± 10% intervals. Except the SM tested against E. coli and Shigella sp., TC, CM tested against Shigella sp. as determined by disk diffusion method i.e. SM demonstrated 39.13%, 52.17% susceptible to E. coli obtained using BBL, local disk respectively testing by the disk diffusion method. In the modified broth disk method, the marked differences between the percentage susceptibility obtained using two disk types were demonstrated with TC, CM tested against Shigella sp. i.e. Reading at 3, 6, 8, 18 hrs., the percentage susceptibility against Shigella sp. were 28.57%, 28.57%, 28.57%, 28.57% obtained using CM-BBL disk; whereas 7.14%, 7.14%, 7.14%, 7.14% obtained using the local disk. The variation in percentage susceptibility obtained using the two disks might be partly from the deviation of actual antibiotic potency from that stated in disk, also the handling and storage procedures provided for each disks (23, 28).

3. Determination of MIC by broth dilution and agar dilution method

All strains of Salmonella sp., Shigella sp., E. coli, V. Cholerae and V. parahaemolyticus demonstrated the identical MIC's or varied by

It was known that PM diffuse poorly into the agar media, so the percentage susceptibility tested against PM using disk diffusion method might have been varied and usually lower than those obtained from other MIC dilution methods (2, 52). Although, the percentage susceptibility of Salmonella sp., Shigella sp., E. coli and V. parahaemolyticus to PM using modified broth disk method demonstrated some agreement with only those obtained using MIC methods. For example, Salmonella sp. showed 55.10%, 95.92%, 100% and 100% susceptible to PM obtained using disk diffusion, modified broth disk, broth dilution and agar dilution method respectively. However, it could be summarized that the modified broth disk method reading at the 6 hrs. interval may be applied as the antimicrobial susceptibility technique for Salmonella sp., Shigella sp., E. coli and V. parahaemolyticus tested against PM also.

Although, it is customary to report organisms in a clear-cut fashion as sensitive or resistant according to the sensitivity test result. However, these terms describe the behaviour of the strains in vitro, it is clearly implied that the results are an indication of the likely therapeutic response (56). To be of real value, the results of laboratory tests would need to be correlated with the therapeutic effect and pharmacology of antibacterial drugs in clinical practice. In some laboratories the MIC (and/or MBC) of organisms are determined by the MIC dilution methods in which the MIC's of individual antibiotic to specific organisms will be obtained. The dosage of administration will be calculated in order to achieve the concentration attained in blood equal or slightly more than the indicated MIC values.

one or two dilutions when tested by the agar dilution and broth dilution method. The one or two dilution differences of the obtained MIC's determined by the two methods were very common and not quite significant (2). Also, the overall data demonstrated that the comparison given in Table 18-21 and Fig. 3-6 showed a slightly higher readings obtained by the broth dilution method. The linear relationship between the MIC's obtained from the two methods shown in Fig. 3-6 clearly demonstrated that any strain with, for example, the MIC of 1.56 µg/ml as determined by broth dilution technique would, majority, got the MIC of 1.25 ug/ml determined by agar dilution method. This was because the broth dilution method end points generally higher than the agar dilution MIC (2).

The percentage susceptibility, interpreted from the individual MIC using Table12, obtained testing the two dilution methods were quite similar. As shown in Table 22 Salmonella sp. demonstrated 66.67% susceptibility to SM as determined by broth dilution method, and 63.33% susceptibility to SM as determined by agar dilution method.

The agreements in percentage susceptibility obtained using the four methods were demonstrated with Salmonella sp., Shigella sp., E. coli, V. parahaemolyticus tested against TC, CM, PN, AM and also demonstrated with Shigella sp. tested against KM.

The agreement in the percentage susceptibility obtained using disk diffusuin method, modified broth disk method using BBL and local disk broth dilution method and agar dilution were demonstraed in Shigella sp. for instance, as 78.57%, 71.43%, 75.00%, 71.43%, 73.33% and 73.33% respectively susceptible to KM.

For technical reasons, many laboratories may find it difficult at present to perform quantitative tests in every case , but this procedure appears mandatory when the results of the test bear on the management of a serious infection (56). The development of simple technique i.e. the modified broth disk method suitable for small as well as large laboratories seems capable of part solution.

The MIC value and the sensitivity results demonstrated the local drug resistant pattern of which all clinicians have to be aware of when selecting any choice of antibiotic therapy. Comparing the MIC's given in Table 18-21 and the MIC's given by the Garrod, L.P. et al (41) in Appendix IV. The data demonstrated the higher MIC's obtained from this study of which reflect the drug therapy ie the conventional Chloramphenicol therapy for Salmonellosis is no longer 100% effective nowadday.