The coryneform bacteria, the mycobacteria and the nocardia along with a few groups of organisms, and even sometimes the streptomycetes are taxonomically quite interrelated, and taxonomic distinction between them is often rather difficult; for this reason, the position of these different genera, and members within each, has undergone repeated changes. All these have very close similarities which involve a very large number of biological characters, although some of the nocardioform, streptomycetes and rhodococcus groups have marked ability to form mycelia, branching often at right angle and producing arthrospores and blastospores; these were distinguished from the mycobacteria because of lack of such conspicuous branching and formation of the spores. Moreover, mycobacteria are almost invariably aerobic, whereas, other members of the nocardioform bacteria like nocardia, rhodococcus etc., vary from aerobic to microaerophilic, many of which are more microaerophilic than aerobic.

Prauser (1967) was the first to coin the term 'nocardioform', and included a variety of species and genera within the nocardioform group in which he chose the 'Nocardia' as the prototype member of this group; this was like the word coliform, represented by Escherichia coli as a prototype of the coliform group which encompasses large number of species and genera within it. Prauser's designation of the nocardioform bacteria has been accepted as a convenient taxonomic platform or a common cover to designate a variety of ill-defined bacteria whose interrelationship and separations are often questionable.

The concept of nocardioform organisms was accepted by Sneath et al (1986) in the Bergey's Manual of Systematic Bacteriology. Chakrabarty et al (1986) and subsequently other workers used the concept of nocardioform bacteria in characterising the nocardia-like
group of organisms isolated from leprosy derived human and animal tissues.

Besides the biological similarities of the bacteria constituting the nocardioforms, from the clinical points of view also, these have very close similarities of the diseases produced by pathogenic actinomycetes e.g. mycobacteria and nocardia in having a widespread occurrence and a generalised distribution, reminiscent of the actinomycotic features of these diseases. Another important similarity observed was as regards the special problems of chemotherapy and antibiotic therapy posed by all these organisms, possibly because of their sporulating branching mycelia, slow growth rate, together with the formation of mycolic acid by most of the organisms. Like the streptomycetes which are producers of many different kinds of antibiotics, and possess, on account of this, multiple antibiotic resistances, many nocardioforms similarly possess multiple drug resistance mediated by R-plasmids. In all these respects, these differ remarkably from most other types of bacteria including the Gram positive bacteria, a class to which they all belong. The slow growth and sporulation in them, associated with long generation time, enable them to resist antibiotic and chemotherapeutic action to a very large extent and these create several types of problems of antibacterial therapy. Thus, firstly, the drugs have to be continuously administered for long periods because of much longer generation time of these bacteria. Secondly, the slow-growing organism can resist the antibiotic and chemotherapeutic action more than the fast-growing organisms because of greater scopes of inactivating the antibiotics by their enzymes. Thirdly, the cell-wall of these bacteria including its mycolic acid is believed to offer obstruction to the entry of the chemotherapeutic agents. Fourthly, many of these organisms are able to sporulate i.e. exist in state of refractoriness to antibiotic
action, as a result of which most of the antibiotics and chemotherapeutic agents cannot act on these spores; finally, these slow-growing bacteria have greater chances of producing mutants to the drugs during the period of prolonged chemotherapy.

The problems of in vitro resistance and concomitant clinical chemotherapy call for the necessity of finding out newer drugs singly, or in various combinations, which could eradicate infections by these bacteria and prevent emergence of drug-resistance; it follows, therefore, that the drug combinations should be effectively additive or preferably synergistic and it should be possible to use these over long periods without significant toxic effects.

Another aspect of these bacteria from the point of view of application of these drugs, is that many of the bacteria develop drug-dependence e.g. streptomycin-utilising mutants of M. tuberculosi. This may happen with respect to some other members of the nocardioform bacteria, although this had not been studied in a manner similar to that of the tubercle bacilli.

Another probable cause of drug-resistance is the production of a variety of β-lactamases, many of which are not amenable to the inhibitory action of clavulanic acid, sulbactam, and such other β-lactam inhibitors.

A point which had been considered to be potentially significant in the chemotherapeutic and antibiotic management of these bacterial infections is of a change-over from normal forms to the cell wall defective L-forms which makes most of the β-lactam antibiotics inactive on them.
Finally, the intracellular location of many of these nocardioform bacteria gives them sanctuary in that many antibiotics do not enter the intracellular milieu at concentrations which are available in the extracellular fluid or in blood; whether they may or may not enter intracellular milieu, their concentrations are often at levels much lower than would be needed to be therapeutically effective. Taking into consideration the fact that even when a drug enters the intracellular site, presumably at a much reduced concentration, a further fraction of it will be bound down to the intracellular proteins and other substances and thereby remain unavailable for intracellular antimicrobial activity.

Thus, because of difficulties of having suitable number of drugs which could act on different members of the nocardioform bacteria after the model of chemotherapy of the tubercle bacillus, there is a need for continued search for newer type of chemotherapeutic agent and antibiotics, and of gainful combinations between them which would give additive and preferably synergistic effects. Such a research seem to be extremely relevant in the field of clinical therapy concerning the nocardioform bacteria as a class. The search for chemotherapeutic and antibiotic agents in the control of mycobacteriosis and nocardiosis has continued for a very long time and although some of the drugs are highly effective, still, the situation in which these drugs find themselves with respect to these organisms is far from satisfactory in comparison with their counterparts seen e.g. in infections with enteric bacteria, staphylococci and streptococci etc.

Although there are continuous reports on newer drugs, a beginning in a new direction was made when Dastidar, Chakrabarty and their coworkers started looking for antimicrobials among the non-conventional antimicrobics such as those found in the conventionally
characterised (in the pharmacopoeae) drug e.g. antihistamines, anti-hypertensives, analgesics, sedatives, as well as, a variety of similar drugs which are widely used in clinical medicine not as antimicrobials but for some other purposes.

Since 1976, these groups of workers have persistently undertaken studies on groups of chemotherapeutic agents, and these include ambodryl (Dastidar et al, 1976) chlorpromazine (Sidney and Raffel, 1960), Promazine (Dash et al 1977), methyl-DOPA (Dastidar et al, 1986), Promethazine (Nyogi, 1988; Kristiansen and Mortensen, 1986), methdilazine (Chattopadhyay et al, 1988), and a large number of such drugs, of which several possess conspicuous antimicrobial properties. As a result of discovery of these new classes of antimicrobics among the non-conventional antimicrobial drugs used for different purposes, it has been possible to test them for simple additive or synergistic effects when combined with respect to each other as well as with respect to a large number of antibiotics tested in a similar manner. All of these antimicrobial effects have been detected first in vitro and subsequently in the standard in vivo models developed for this purpose (inbred Swiss strain of white mice). It has been found that some of the combinations with respect to enterobacteria or Gram positive organisms like the Bacillus spp. or streptococci and staphylococci had on some occasions synergistic effects, (Ray et al 1986; Chakraborty et al, 1987; Chattopadhyay et al, 1988) yet on other occasions just simple additive effects, and in still other cases, antagonistic effects were seen. In all these respects these compounds behaved like the classical antimicrobial agents.

Table 2.7 shows the list of such drugs studied by Chakrabarty, Dastidar and their colleagues together with a list of similar drugs which have been reported by others working on similar
lines. The nonconventional antibiotics and chemotherapeutic agents already appeared to be promising, and these new groups of compounds have attracted the attention of the scientists all over the world; it is interesting to note that an international conference on these classes of nonconventional chemotherapeutics is going to be held in May, 1990 at Copenhagen, Denmark specially to discuss the multifaceted problems that the workers of this group have raised. This highlights the potential importance of these groups of compounds. Although studies on this important emerging group of antimicrobials have begun, so far there are very few reports or actually none in most instances on the activity of these drugs towards the mycobacteria and nocardia groups, and in general, on other members of the nocardioform bacteria. In this respect the report of Meindle and Miroslnava (1987) is worth remembering, as they suspected histamine as a growth promoting factor in different tubercular strains, thus explaining the antimycobacterial property of methdilazine. Chattopadhyay, working on this problem has found that histamine favours the growth of _B. sublitis _and _E. coli _and addition of methadilazine inhibited their growth (unpublished data).

There is, therefore, a paucity of adequate number of drugs which are effective on different mycobaterial and nocardial infections and that these newly emerging group of nonconventional antimicrobics can be looked for a probable activity on these bacteria which is the chief reason for which our studies were designed in respect of mycobateria and nocardia group of organisms for evaluating their _in vitro _and _in vivo _role.

The activities of some of these new nonconventional antimicrobics and chemotherapeutic (NAC) agents need to be discussed; thus using _m-DOPA _as a NAC agent it was found that not only it inhibits
large series of Gram positive and Gram negative organisms, but 5 atypical variants of the 14 standard strains of mycobacteria tested in KSM also showed inhibition at a concentration of < 200 μg/ml, whereas the minimum inhibitory concentrations with respect to rest of the bacteria in KSM, and with respect to all the strains in LJM were beyond 200 μg/ml. Another interesting finding is the excellent suitability of KSM as a test sensitivity medium due to the absence of drug inactivating proteins, compared with LJM.

The above results of preliminary screening show that m-DOPA may be used as a starting material for further synthesis of derivative compounds, hopefully with increased antibacterial and reduced anti-hypertensive effects, and may prove to be a prospective agent for further chemotherapeutic application in mycobacteriosis.

Further, while m-DOPA was combined with TMP, a synergistic effect was observed not only with respect to a large series of Gram positive and Gram negative strains, but also with respect to mycobacterial test strains. The synergism observed appeared to be significant in nature and showed that it obeys the laws of the chemotherapeutics.

Another aspect of the sensitivity of mycobacteria was with respect to augmentin which consists of a fixed concentration of clavulanic acid with amoxycillin. It has been found that augmentin could inhibit most of the mycobacteria at a concentration of 0.25 μg/ml. but the replacement and/or addition of carbenicillin or cephalaxin or cloxacillin in place of or with amoxycillin in a suitable concentration did not alter the picture of mycobacterial sensitivity, indicating that the amoxycillin and clavulanic acid combination is probably one of the best achievable combinations.
The same results were obtained when tube dilution tests were carried out in parallel with tests on solid medium. However, when augmentin was combined with established antitubercular agents like INH, streptomycin, ethambutol or rifampicin by colony counting method (Oberhofer, 1985), it was found that these combinations were successful in delaying the appearance of resistant mutant colonies by several weeks in almost all the mycobacterial strains which may appear to be an information of sufficient clinical significance.

On the other hand when augmentin was combined with other established antinocardial agents like trimethoprim, trimethoprim-sulfamethoxazole or dapsone, no synergistic effect was noted against any of the strains of nocardiae.

Following the interesting finding of antibacterial property of methdilazine with respect to the non-mycobacterial bacteria (Chattopadhyay et al, 1988) we have found remarkable activity towards mycobacteria and most of these strains were inhibited at a concentration of < 12.5 μg/ml excepting the strains of M. smegmatis. As an extension of this study, when methdilazine was combined with other established antitubercular agents like streptomycin and rifampicin, it was found that methdilazine in combination with the streptomycin produced an indifference of effects whereas with rifampicin, produced antagonistic effects.

However, the picture was rather different when a large number of chemotherapeutic agents and antibiotics like streptomycin, kanamycin, erythromycin, trimethoprim-sulfamethoxazole, doxycycline, rifampicin, augmentin, metronidazole and dapsone and several antitumour agents like cytosine arabinocide, 6-mercaptopurine, vincristine, mitomycin C, methotrexate and antihistaminic
methdilazine were tested against the nocardioform organisms isolated from leprosy tissues. All of them showed remarkable resistance and a combination of cationic detergent antibiotic polymyxin B with mitomycin C offers no advantages over the agents used alone.

To correlate the in vitro efficacy of augmentin against M. marinum in an in vivo system, mouse footpad was chosen as a model in which it was found that augmentin provided significant protection as evidenced by the objective measurement of mouse footpad thickness and the number of bacteria recovered from them after producing inflammation by inoculation of M. marinum. Similarly, the in vivo effect of methdilazine was evaluated against M. tuberculosis H37 Rv strain by producing systemic infection in mice and protecting them by methdilazine through intraperitoneal route, and it showed a significant modification of the postmortem macroscopic and microscopic findings as well as bacterial recovery in the treated group (10 μg/gm of body weight/day) compared with untreated one. It was interesting to note that nocardioform bacteria comprising mycobacteria, nocardia and related species of organisms, which possessed spectre of multiplicity of drug resistance when tested against a number of these newly discovered chemotherapeutic agents both in vitro and in vivo, showed some degree of sensitivity. The agents also could act individually, or in combination, producing synergistic, indifferent or antagonistic actions.

The mechanism of drug resistance in mycobacteria with respect to β-lactam antibiotics producing β-lactamases had not been studied very well and understood as yet. Since clavulanic acid per se has very little antibacterial property (Cooper, 1980), antimycobacterial action of augmentin in contrast to amoxycillin towards almost all the strains of mycobacteria, suggests that the presence of
β-lactamase is the cause of β-lactam resistance in these bacteria and that it can be countered by clavulanic acid and allow various β-lactams to act. Until complete physical and chemical characterisation of these β-lactamases is available, the most effective enzyme inhibitors cannot probably be used. However, the present study shows clavulanic acid could be combined favourably with several established antitubercular agents such as streptomycin, rifampicin, INH and ethambutol, delaying the emergence of resistant mutants. It appears to be of considerable clinical significance as far as chemotherapy of mycobacteriosis is concerned. Application of these data to clinical isolates can throw light on how far this information may have practical application.

The same hopeful picture was not seen in the case of the nocardioform bacteria derived from leprosy tissue which were found to be resistant to most of the antimicrobics, including antibiotics as well as to antitumour agents, and the use of cationic detergent polymyxin B in combination with mitomycin C to help the entry of the antibiotics inside the cell did not help. It shows that the mechanism of drug resistance of this group of nocardioform organisms is probably different from that of mycobacteria. It can be accounted for by the formation of mycelia which could sporulate into blastospores and arthrospores rendering these inaccessible to the drug entry and the long period of germination coupled with some inherent mechanisms of drug resistance, may be other contributing factors. Therefore, the search should be continued for the effective chemotherapeutic agents from these nonconventional group of antibiotics along with their combined effects with antibiotics and established chemotherapeutic agents in various possible combinations. A beginning only in this direction had been made, but we may hope to discover newer effective drugs as well as the newer modes of action that these drugs will involve.