Unlike tryptophan, alanine is not much effective against DMS, although it is considerably effective against EMS. But like tryptophan, in case of alanine also, the duration of pre-treatment made considerable difference. While a 10 h pre-treatment was simply ineffective against DMS, the same concentration at longer duration, i.e. 20 h pre-treatment could considerably reduce chromosomal aberration. Similar observations regarding greater efficacy of antimutagen following pretreatment for longer duration like 20 h were reported by Abutalybov *et al.*[7] working with *Allium fistulam* using ionol and Alekperov[15] working with *Crepis capillaris* using α-tocopherol as antimutagen. It appears that different antimutagens are not equally effective against various mutagens. But an ideal antimutagen should be the one which is universally effective against all mutagens or at least a broad spectrum of mutagens. Tryptophan deserves further and detailed investigation to ascertain whether it can be called a broad spectrum antimutagen or not. Apart from a chromosomal aberration test in onion root tip, the efficacy of antimutagens has been tested in the prokaryotic system, viz. *Salmonella typhimurium* by Bala and Grover[8], Grover and Bala[9] using citrus and myroblan fruit juice as source of antimutagen which are known to be very rich in ascorbic acid. Antimutagenic activity of polyphenols like caffeic acid and gallic acid has been demonstrated by Chan *et al.*[10] using the Salmonella test system.

Apart from tryptophan and alanine, at least another amino acid, cystein is known to have antimutagenic effect as demonstrated by Garina and Nurzhanova[11] and Ionaitis Sokolov and Ranchyalis[12]. It is not understood how a simple amino acid molecule can protect the chromosomes against mutagenic treatment damage. Kada *et al.*[13] classified antimutagens into two major groups: desmutagen and bioantimutagen. Desmutagens are defined as those which inactivate mutagen *in vitro* before they reach the interior of cell. It is likely that antimutagens like tryptophan and alanine neutralize the free radicals generated by the mutagens, thereby rendering them ineffective. Strelchk[14] detected antimutagenic activity of the extracts of *Eleutherococcus senticosum* against EMS by post treatment. However the extract had a protective effect only in the first four hours after post treatment. Obviously the mutagens do all the damage in the first four hours after treatment and therefore post treatment with antimutagen beyond this period is of no avail.

The present study clearly shows that apart from tryptophan, another amino acid alanine also has antimutagenic property, although tryptophan is superior to alanine as antimutagen.

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Anti-staphylococcal activity of *Pseudomonas aeruginosa*

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Anti-staphylococcal activity of *Pseudomonas aeruginosa* was studied on a total of 118 strains of staphylococci. The results revealed the existence of a highly active anti-staphylococcal *Pseudomonas aeruginosa* metabolite which appears to be pyocyanine. It was even active against methicillin resistant *Staphylococcus aureus* (MRSA) and coagulase negative staphylococci (CONS).

PSEUDOMONAS represents the major group of non-differentiating microorganisms producing antibiotics[1]. The practical use of antibiotics from *Pseudomonas* sp. dates back to the period before the antibiotic era. Emmerich and Low[2] reported that the cell-free culture fluid of *Pseudomonas aeruginosa*, concentrated to one tenth of its original volume, killed several kinds of bacteria. This was extensively used in the therapy of diphtheria, influenza and meningitis during the first two decades of the century[1]. During the antibiotic era, approximately 50 different antibiotic substances from *Pseudomonas* sp. were discovered. Of these compounds, pyocyanine and pyrrolnitrin were most abundantly present. We failed to find in literature any study regarding anti-staphylococcal activity of *P. aeruginosa*, except a report on the biosyn-

thesis of batumin[3], especially on methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase negative staphylococci (CONS).

Methicillin-resistant *Staphylococcus aureus* (MRSA) emerged in 1980 as a major clinical and epidemiologic problem in hospitals[4]. It was suggested that hospitals of all sizes were facing the MRSA problem[5]. The coagulase negative *Staphylococcus* sp. (CONS) as a group constitutes a major component of the normal microflora of the human. Over the last two decades, there has been an increase in the documentation of infections due to CONS, especially in hospitalized immunocompromised patients and in patients having prosthetic and indwelling devices[5].

In this study, we evaluated 103 strains of *S. aureus* and 15 strains of coagulase negative staphylococci for their susceptibility to metabolites of *P. aeruginosa* by testing against 80 *Pseudomonas* strains. In addition, attempts were also made to identify and characterize the active metabolites of *P. aeruginosa*.

A total of 80 *P. aeruginosa* strains isolated from a variety of clinical specimens were identified by conventional tests described elsewhere[6]. Of these 80 strains, 75 were pyocyanine producers and 5 were non-pyocyanine producers. The strains were maintained in nutrient agar slants and stored at 4°C and subcultured once in every 15 days.

A total of 103 strains of *S. aureus*, including 70 methicillin sensitive *S. aureus* (MSSA), 30 methicillin resistant *S. aureus* (MRSA) isolated from a variety of clinical specimens, one strain each *S. aureus* NCTC 6571, *S. aureus* Cowan I and *S. aureus* Plazen were maintained in soft nutrient agar butts at 4°C. The methicillin susceptibility of these strains was tested by the methods described elsewhere[7]. Fifteen strains of coagulase negative *Staphylococcus* sp. isolated from a variety of clinical specimens, were maintained in soft nutrient agar butts.

Anti-staphylococcal activity of *P. aeruginosa* was tested using (i) pyocin typing like method and (ii) agar overlay method.

In pyocin typing like method, a young peptone water culture of *P. aeruginosa* adjusted to 0.5 McFarland turbidity standard was inoculated in the centre of a dry nutrient agar plate as a line of 4 mm width using a bacteriological loop. The plates were incubated at 37°C for 18–24 h. Then the growth was gently scrapped with an 'L' shaped thin glass rod. The remaining organisms were spread over the agar surface with a cotton swab soaked in saline. The plates were exposed to UV light in a micro-titre plate sterilizer for 5 min. (ref. 8). After killing *Pseudomonas*, young peptone water culture of staphylococci, adjusted to 0.5 McFarland standard, was inoculated as 4 mm width lines at right angles to the original *Pseudomonas* inoculum. The plates were incubated at 37°C for 18–24 h and scanned for inhibition of growth of staphylococci over original *Pseudomonas* inoculum[8].

In the agar overlay method, 6 strains of *P. aeruginosa* were inoculated into dry nutrient agar (NA) plates (8 cm diameter) as spots by transferring 10 µl of inoculum prepared as explained above. The plates were incubated at 37°C for 18–24 h. The growth was scrapped and the plates were exposed to UV as described above. These plates were overlaid with 10 ml of sterile molten agar at 45–50°C to which 25 µl of staphylococcal inoculum was added and mixed. The plates were incubated at 37°C for 18–24 h and scanned for the zone of inhibition of growth over the *Pseudomonas* inoculum spots.

The effect of presence of oxygen on antibiosis was assessed by growing 10 pyocyanine-producing strains of *P. aeruginosa* on nutrient agar supplemented with 0.1% KNO_3 (ref. 9) and kept anaerobically in a commercially available gas pack system. The growth was scrapped and exposed to UV light and anti-staphylococcal activity was tested as described above.

The effect of iron in the medium on antibiosis was tested by incorporating iron ($FeCl_3$) at 50, 100, 150, 200, 250 and 300 mg/l separately into nutrient agar[10]. The anti-staphylococcal activity was assayed as described above.

With both the methods, all *S. aureus* strains including MRSA and CONS were found to be susceptible to pyocyanine produced by *P. aeruginosa*. The inhibition zone was directly proportional to the bluish-green discolouration on the plate. While none of the 5 strains of pyocyanine non-producing *P. aeruginosa* showed any activity on staphylococci, *P. aeruginosa* grown anaerobically failed to inhibit the growth of staphylococci, tested. Incorporation of iron into the medium did not affect the susceptibility of *S. aureus* to *P. aeruginosa*.

This study clearly shows the presence of some metabolite(s) of *P. aeruginosa* which is highly active against staphylococci. The most promising finding is that, all the 30 strains of MRSA tested were found to be susceptible. The possibility of depletion of nutrients by the *Pseudomonas* strains from the medium as a reason for its anti-staphylococcal activity is ruled out as the agar overlay method showed absolute agreement with the results of pyocin typing like method. Pyocyanine production showed a positive correlation with the anti-staphylococcal activity of *P. aeruginosa*. *P. aeruginosa* grown in the absence of oxygen failed to exhibit anti-staphylococcal activity. Since pyocyanine is an oxygen-dependent pigment, this observation suggests the role of pyocyanine in the anti-staphylococcal activity of *P. aeruginosa*. Kumar and Bezbaruah[10] reported that siderophose produced by the *Pseudomonas* had a vital role in its anti-fungal activity. Our studies on the other hand, showed that siderophose is not responsible for its anti-staphylococcal activity.

In conclusion, pyocyanine-producing *P. aeruginosa* exhibits potent anti-staphylococcal activity even on MRSA and CONS strains. The active substance appears to be pyocyanine. This may be exploited for the development of an effective anti-staphylococcal agent.

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Community structure of larval trematode fauna of the snail *Thiara tuberculata* from a freshwater stream at Visakhapatnam, Andhra Pradesh

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Monthly samples of the snail *Thiara tuberculata*, collected from a freshwater stream at Visakhapatnam, were examined for larval trematode infections. Cercarial infections occurred in 38.2% of the snails. Nineteen species of cercariae belonging to 10 families were found, with xiphidiocercariae as dominant members. Fifteen species of cercariae belonged to allogenic category and four species were autogenic. Four species are identified as having zoonotic potential with fish serving as second intermediate hosts. No seasonal pattern is exhibited in the overall prevalence of infection and the community diversity.

THE freshwater bodies of tropical countries harbour dense populations of snails that serve as intermediate hosts for a wide range of digenetic trematodes infecting various groups of vertebrates including man. Investigations dealing with analysis of larval trematode fauna of snails, their transmission patterns and host–parasite relationships are receiving considerable attention. From the Indian region, studies relating to larval trematode fauna of snails have received attention ever since the publication of the monumental work by Sewell[1]. The approach so far has been taxonomic in nature. Information available on ecological aspects of parasitism in snails is very scanty.

In recent studies, much emphasis has been given to investigations dealing with analysis of community structure of larval digenean infections in snails[2–8]. The community with reference to parasites has been defined as the complex assemblage of parasites occurring within a host. Terms such as infracommunity, component community and compound community are in use, to define various heirarchical levels, the infracommunity to include all the parasites in a single host, the component community including all the parasites in a single population of hosts and the component community denoting all the parasite communities within an ecosystem[2]. The parasite community within a host population is considered as complex and a result of continuous interaction of host–parasite and environment. Its species composition and diversity are under the control of a complex set of biotic and abiotic factors which have direct impact on the transmission patterns. An analysis of this relationship is expected to be useful in understanding the forces which operate in structuring the parasite communities.

In the course of our studies on larval trematode infections of freshwater snails of Visakhapatnam region, it was found that the snail *Thiara tuberculata* in a local freshwater stream serves as a suitable host for a wide range of larval digeneans. An investigation has, therefore been undertaken during 1992–1994, to study the organization of larval trematode fauna of the snail including the species composition, their diversity, dominance and seasonal changes.

The study area is a small freshwater stream originating from Mehadrigedda reservoir flowing in a north-easterly direction, to ultimately join the creek near a fishing harbour. The snail *T. tuberculata* forms dense populations in the steam throughout the year. The collection spot located adjacent to the reservoir is surrounded by mango trees on which nestle a number of resident birds like pond herons, egrets and kites. Ducks are also common. Domestic animals like sheep and cattle visit the area.