Psychotropic drugs inhibit swarming in *Proteus* spp. and related genera


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**Objective:** To study the in vitro activity against *Proteus* and *Morganella* and the swarming inhibition capacity of 10 psychiatric drugs (amitriptyline, clomipramine, imipramine, maprotiline, chlorpromazine, sertraline, promethazine, fluphenazine, diazepam, and dimenhydrinate).

**Methods:** MIC determinations on Mueller–Hinton agar by NCCLS methods and observations of subinhibitory concentrations for effects on swarming.

**Results:** All the drugs showed a very low inhibitory activity (MIC$_{90}$ range: 512 mg/L to >512 mg/L.). Amitriptyline and imipramine partially inhibited the swarming at subinhibitory concentrations. Clomipramine, sertraline, maprotiline, promethazine, and chlorpromazine totally inhibited swarming at subinhibitory concentrations. Sertraline had the highest anti-swarming activity (32 mg/L for all the strains). Fluphenazine, diazepam and dimenhydrinate did not show any affect on the swarming phenomenon.

**Conclusions:** Some psychotropic drugs, and mainly some selective serotonin re-uptake inhibitors, such as sertraline, are able to inhibit swarming in *Proteus* and related species. This behavior may be interesting for diagnosis purposes, and because swarming seems to be related with pathogenicity in these species, and might be an alternative target against these bacteria.

**Key words:** *Proteus*, non-antibiotic drugs, psychotropic drugs, swarming.

**INTRODUCTION**

*Proteus* and related genera (*Morganella, Providencia*) [1,2] are pleomorphic, Gram-negative bacilli, easily recognizable in non-selective media, such as blood agar, by the swarming phenomenon. They form part of the bowel normal flora, and are opportunistic pathogens, mainly causing lithiasis-related urinary tract infection (UTIs).

The swarming on agar plates (in broth they grow with a filamentous aspect that is considered to be equivalent to the swarming) [3] is related to the great mobility of cells, and frequently impairs the isolation of colonies and the identification of other bacteria in mixed infections. Culture media have been developed that reduce or suppress this phenomenon, such as CLED or MacConkey agar. Several chemicals have been also described that inhibit swarming, such as p-nitrophenyl-glycerol, boric acid, some dithiocarbamates and other chelators [4], triclosan [5], salicylates [6], surfactant agents [7] and dyes such as acridine orange [8]. Recently, there have been reports [9] of some drugs (promethazine, imipramine and chlorpromazine isomers) that act as inhibitors of swarming at subinhibitory concentrations. Moreover, swarming has been related to the pathogenic capacity of the bacteria [9].

We have studied the in vitro activity of 10 psychotropic drugs and drugs structurally related, though mainly used as antihistamine drugs, against *Proteus mirabilis, P. vulgaris* and *Morganella morganii*, and their effect on swarming in these species.
MATERIALS AND METHODS

Drugs
Three phenothiazines (chlorpromazine, fluphenazine, promethazine), four tricyclic or heterocyclic antidepressants (amitriptyline, clomipramine, imipramine, maprotiline), one selective serotonin reuptake inhibitor (sertraline), one benzodiazepine (diazepam) and one antihistamine drug (dimenhydrinate) were provided, as standard powder, by their respective manufacturers.

Strains
Thirty-two clinical isolates of P. mirabilis, 32 of P. vulgaris and 32 of M. morganii, most of them isolated from UTIs, were used.

MICs were performed by the agar dilution method, according to previously described methods [10]. For measuring swarming, a 10-μL drop from a McFarland 0.5 inoculum (approximately 10^6 CFUs) was plated on a Mueller-Hinton agar plate and on Mueller-Hinton agar plates with double dilutions of every drug tested, between the MIC and 1 mg/L. We incubated plates for 18 h, and then we measured the diameter of the swarming area. We considered partial inhibition of swarming to have occurred when the swarming diameter with the drug was ≤50% of the swarming diameter without the drug. We considered total inhibition to have occurred when there was no swarming or there was a faint haze around the growth area.

RESULTS

As a rule, the antimicrobial activity of the drugs tested was low, MICs ranging between 512 mg/L and >512 mg/L (Table 1). However, some of these drugs showed a surprising capacity to inhibit swarming (Table 1). Tricyclic and heterocyclic antidepressants had different capacities for inhibiting swarming, though the four drugs are very similar structurally. Two tricyclic antidepressants, amitriptyline and imipramine, were the only drugs that showed partial inhibition of swarming. They partially inhibit the swarming at concentrations 2–4-fold lower than MICs. The other tricyclic (clomipramine) and heterocyclic (maprotiline) antidepressants did not show partial inhibition, but they inhibited swarming totally at concentrations 2–4-fold lower than MICs. The only bicyclic antidepressant used, diazepam, a benzodiazepine, did not show any inhibitory activity on growth and on swarming. The behavior of phenothiazines against swarming was heterogeneous, though structurally they are similar. Fluphenazine did not have any activity, while promethazine and chlorpromazine totally inhibited swarming at concentrations 2–8-fold lower than MICs. Chlorpromazine was the most active phenothiazine, inhibiting swarming in all cases at 128 mg/L. Dimenhydrinate did not show any activity on the swarming phenomenon. Sertraline, the only selective serotonin reuptake inhibitor tested, had low inhibitory activity on bacterial growth, though it was the most active drug tested, MICs ranging between 64 and 512 mg/L. This drug inhibited swarming at 32 mg/L in all cases. Its remarkable that, for all the drugs, concentrations inhibiting swarming were the same for all the strains, independent of their MICs.

DISCUSSION

Swarming is a characteristic of some enterobacteria, such as Proteus, Morganella and Providencia. The presence of flagella seems to be closely linked with swarming, as well as with virulence [11,12]. Ascending UTIs have been shown to be more frequent in swarming strains [13]. There is also evidence of a greater invasive capacity in the urinary tract epithelium in strains that show swarming [14]. Moreover, the urease capacity is constitutively expressed in strains that present swarming, but not in strains lacking it, in which it can be inducible [15].

Table 1 Inhibition of growth and swarming in Proteus and Morganella by psychotropic drugs

<table>
<thead>
<tr>
<th>Drugs</th>
<th>MIC (mg/L)</th>
<th>Swarming inhibition (mg/L)</th>
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<tr>
<td></td>
<td>50</td>
<td>90</td>
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<tr>
<td>Amitriptyline</td>
<td>512</td>
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<td>Clomipramine</td>
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<td>Imipramine</td>
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<td>Maprotiline</td>
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<td>Promethazine</td>
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<td>&gt;512</td>
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<td>Chlorpromazine</td>
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<td>&gt;512</td>
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<td>Fluphenazine</td>
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<td>Sertraline</td>
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<td>Diazepam</td>
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<td>Dimenhydrinate</td>
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Some swarming strains also produce large quantities of slime, which might be important in promoting the close contact between bacteria that occurs during this phenomenon, as has been shown in *Escherichia coli* and *Salmonella typhimurium* [16]. The antimicrobial activity of psychotropic drugs has been shown previously [17–19], and the effects of some other psychiatric drugs on swarming have also been previously reported [8]. In our study, the most remarkable anti-swarming activity was shown by some phenothiazines, such as chlorpromazine and promethazine, and especially by a selective serotonin reuptake inhibitor, such as sertraline. Sertraline acts, in humans, as an inhibitor of mechanisms that take serotonin from the synaptic space to the presynaptic neuron, thus increasing the concentration of serotonin in the synaptic space. We have previously shown that serotonin has a surprising inhibitory activity on some Gram-positive bacteria [19]. Serotonin seems to interfere with other metabolic or physiologic bacterial mechanisms too, leading to this kind of modification in the behavior of swarming bacteria.

Serotonin is formed by a bicyclic structure being linked to a difluorinated cyclic structure. This kind of cyclic, difluorinated structure has previously been shown to be related to antimicrobial activity in other non-antibiotic drugs, such as diclofenac [20]. Microscopic studies should be useful in determining the morphologic modifications induced in these bacteria. A knowledge of drugs inhibiting swarming [4–8] and of additional factors that modify the anti-swarming activity of these substances (synergy between quinine and omeprazol with promethazine against swarming [8], flagella alterations due to osmotic stress caused by the underexpression of OmpF) [6] is useful, both from a diagnostic point of view and because swarming seems to be an important pathogenic mechanism in these genera. The close relationship between swarming and some virulence factors [21], such as the presence or not of flagella [11], and the possibility that drugs inhibiting swarming might notably reduce the pathogenic capacity of these microorganisms, may make swarming an alternative target for use against these microorganisms. Moreover, if drugs inhibiting swarming at therapeutic concentrations can modify the pathogenic capacity of these bacteria, they may also modify the evolution of infections by these microorganisms when these psychiatric drugs are being simultaneously administered. All these facts warrant new studies on this phenomenon.

**References**