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# Potent antibacterial activity of oligo-3-aminopyridine against *Staphylococcus aureus* and *Enterococcus faecalis*

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Oligo-3-aminopyridine (OAP) was tested for its antibacterial activity against two gram positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and two gram negative (*Escherchia coli* and *Pseudomonas aeruginosa*) bacterial strains. Antibacterial efficacy was measured by disk diffusion assay. Inhibition zones demonstrated that 50 µg OAP showed strong antibacterial activity against the gram-positive strains; the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were found to be 25 µg/ml and 50 µg/ml, respectively. The results indicate that OAP is a potent antibacterial agent against *S. aureus* and *E. faecalis.*

**Keywords**: Antibacterial activity, *S. aureus*, *E. faecalis*, oligo-3-aminopyridine.

The basic mechanisms of antibiotic action against bacterial cells are: (i) inhibition of cell wall synthesis (e.g. penicillins); (ii) inhibition of protein synthesis (e.g. tetracyclines); (iii) alteration of cell membrane (e.g. polymyxins); (iv) inhibition of nucleic acid synthesis (e.g. quinolones); and (v) antimetabolite activity (e.g. sulfonamides)[1]. Inhibition of cell wall synthesis is the most common mechanism of antibiotic action. All cells, including bacteria, also have a cell membrane, which allows in and out of the substances in the cell in a controlled manner. Synthetic compounds may affect the integrity of the cell membrane leading to cell death[2]. Such molecules may also mimic the complex structures having *in vivo* antibacterial activity[3]. Synthetic antibacterial agents form antiseptic ingredients in commercial products, such as liquid soap[4] and also used in *in vivo* systems against bacterial infections[5], but require multiphase clinical trials to be accepted as a commercial drug[6].

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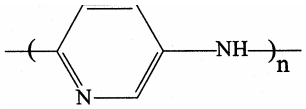


Fig. 1—Structure of oligo-3-aminopyridine

*Staphylococcus aureus* and *Enterococcus faecalis* are the types of strains that can cause serious infections. Although penicillin, methicillin and vancomycin have been used successfully to cure these infections, the resistant strains to these antibiotics have also evolved[7,8]. Thus, one of the long-term measures against these infections is to develop novel antibiotics that are effective against specific strains[9,10]. In this study, we investigated the antibacterial potential of oligo-3-aminopyridine (Fig. 1), synthesized recently by our group[11], against two gram positive (*S. aureus* and *E. faecalis*) and two gram negative (*Escherchia coli* and *Pseudomonas aeruginosa*) bacterial strains.

## Materials and Methods

Mueller-Hinton broth (MHB) and Mueller-Hinton agar (MHA) were from Merck. Blank, gentamicin and vancomycin control disks were from Oxoid. All other chemicals were purchased from Merck with all reagents used being of the highest purity available.

Oligo-3-aminopyridine (OAP) was synthesized[11] from the oxidative polycondensation of 3-aminopyridine with NaOCl and $H_2O_2$ in an aqueous acidic medium at 25°-90°C and characterized by $^1$HNMR, FT-IR, UV-vis and elemental analysis. The number-average molecular wt. ($M_n$), weight-average molecular wt. ($M_w$) and polydispersity index values of OAP were found to be 250, 800 g mol$^{-1}$ and 3.20, respectively, using NaOCl, and 240, 840 g mol$^{-1}$ and 3.50, respectively, using $H_2O_2$. About 90% 3-aminopyridine was converted into OAP. Thermogravimetric analyses showed that OAP was stable towards thermo-oxidative decomposition.

## Bacterial strains and antibacterial susceptibility testing

Four representative strains, Gram-positive *S. aureus* (ATCC 25923) and *E. faecalis* (ATCC 29212) and Gram-negative *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were used.

Table 1 — Antibacterial activity of oligo-3-aminopyridine (OAP) determined by disk diffusion assay
[Values represent the diameter (mm) of inhibition zone produced around each disk and are average of 3 separate experiments]

| Bacterial strain | OAP/disk | | Gentamicin | Vancomycin | Methanol |
|---|---|---|---|---|---|
| | 10 µg | 50 µg | 10 µg | 30 µg | 25 µl |
| | | | Inhibition zones* (mm) | | |
| *E. coli* | NZ | NZ | 23 | ND | NZ |
| *P. aeruginosa* | NZ | NZ | ND | ND | NZ |
| *S. aureus* | <10 | 17 | 21 | 17 | NZ |
| *E. faecalis* | <10 | 20 | 14 | 18 | NZ |

ND, Not determined; NZ, No zone observed; * Includes the diameter of the disk (6 mm)

OAP was dissolved in methanol and two different concentrations (10 and 50 µg/ml) were used in disk diffusion assay, as standardised by NCCLS (National Committee for Clinical Laboratory Standards)[12] to determine antibacterial activity. Briefly, the microorganisms were grown on MHA plates and then 3 ml of MHB was inoculated with 4-5 well-separated colonies for each strain. The concentrations of suspensions of tested microorganisms were adjusted to $10^8$ cells/ml using freshly prepared 0.5 McFarland turbidity standard. Each bacterial suspension was spread over the surface of MHA using sterile cotton swabs. Filter paper disks (6 mm in diameter, Oxoid) were soaked with 10 and 50 µg (in 25 µl of methanol) of OAP and then placed onto the inoculated agar surface. After keeping at room temperature for ~30 min, they were incubated at 37°C for 20 hr. Disks containing gentamicin (10 µg), vancomycin (30 µg) and methanol-only (25 µl) were included as positive and negative controls, respectively. The results were expressed in terms of the diameter of inhibition zones and the presented values are average of three separate experiments.

Broth macrodilution assay was performed, as recommended by NCCLS[12] for determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Bacterial strains were cultured overnight at 37°C and suspended in MHB to obtain a final inoculum density of $10^6$ cells/ml and 0.5 ml of this suspension was added to 0.5 ml of susceptibility test broth containing serial 2-fold dilutions of OAP. Two tubes were also included to check media sterility. All the tubes were incubated at 37°C for 20 hr. MIC was considered as the lowest concentration at which OAP prevented visible bacterial growth.

For MBC determination, 10 µl suspensions from each negative tube and from positive growth control were spread onto MHA plates. The plates were incubated overnight at 37°C and MBC was defined as the lowest concentration of OAP producing no colony[13].

Table 2 — MIC and MBC values of OAP against *S .aureus* as determined by Broth macrodilution assay

| | Concentration of OAP (µg/µl) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 100 | 50 | 25 | 12.5 | 6.25 | 3.13 | 1.56 | 0.78 | 0.39 |
| Growth | – | – | – MIC | + | + | + | + | + | + |
| | – | – | MBC + | + | + | + | + | + | + |

(+), Bacterial growth observed; (-), no bacterial growth observed

## Results and Discussion

In disk diffusion assays, OAP showed strong antibacterial activity against two gram-positive bacterial strains *S. aureus* and *E. faecalis*. However, no inhibition zone was observed against two gram-negative strains (Table 1). While gentamicin (10 µg) produced >20 mm inhibition zone constantly, methanol-only, which was used to dissolve OAP did not produce any inhibition zone against all the strains. 50 µg OAP was more effective against *E. faecalis* and produced an average inhibition zone of 20 mm. The inhibition zones were <10 mm when 10 µg OAP was used per disk, indicating a concentration-dependent antibacterial effect of OAP against gram-positive strains.

Broth macrodilution assay was performed for the determination of MIC. The lowest concentration of OAP that prevented visible bacterial growth was 25 µg/ml in all experiments, while the lowest concentration of OAP inhibiting colony formation on agar surface was 50 µg/ml (Table 2).

The structural differences in the membranes of gram-positive and gram-negative bacteria cause

permeability differences and this might explain the ineffectiveness of OAP against *E. coli* and *P. aeruginosa*. The outer membrane structure in gram-negative bacteria consists of a characteristic lipopolysaccharide-lipoprotein complex and is inaccessible to some antibiotics, including penicillin[1], thus, OAP might have failed to cross the outer membrane in these strains. However, the precise mechanism of antibacterial action of OAP against gram-positive bacterial strains requires to be studied further.

The results indicate that OAP is a potent antibacterial agent against gram-positive bacterial strains *S. aureus* and *E. faecalis* and deserves further studies for utilizing as an antiseptic agent.

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