Comparison of e-test Values for Standard Antibiotics and Conventional Antimicrobial Assay Values for Ethanoic Acids against Nosocomial Multidrug-resistant *Pseudomonas aeruginosa*

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Abstract

The present study aimed to determine the susceptibility of *Pseudomonas aeruginosa* strains isolated from patients with nosocomial infections to standard synthetic chemical antibiotics and organic ethanoic acids derived from local produce. The minimum inhibitory concentrations (MIC) of the standard synthetic antibiotics determined from standard e-test results and antibiotic sensitivity tests showed many multidrug-resistant strains among the isolates. We compared the susceptibility of these strains to organic ethanoic acids derived from different sources using standard microbiological assays. All strains of *P. aeruginosa* isolated from the patients were susceptible to the organic ethanoic acids with a satisfactory MIC and minimum bactericidal concentrations. Therefore, organic ethanoic acids were more effective against *P. aeruginosa* than standard synthetic antibiotics.

Keywords: Antimicrobial activity, *Pseudomonas aeruginosa*, ethanoic acid, Nosocomial infection, Immuno suppressed, Vinegar

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INTRODUCTION

*Pseudomonas aeruginosa* is a Gram-negative motile bacterium that is a major cause of community-acquired, and hospital-borne nosocomial infections. The major concern about nosocomial infections with *P. aeruginosa* is that > 10% of infections are caused by multidrug-resistant species that arise through antibiotic abuse. *Pseudomonas* is only one among many bacterial species that have become resistant to multiple antibiotics, and the most resistant strain is *P. aeruginosa*. Gessard isolated *P. aeruginosa* from green pus in 1882 and since then it has been studied in detail, especially with regard to nosocomial infections. The characteristic virulence factors of this bacterium are the exopigments pyoverdin and pyocyanin, which are potentially involved in the occurrence of these infections. *Pseudomonas aeruginosa* causes versatile infections in humans, especially when immunosuppressed, and it is categorized as the most important bacterial cause of infections acquired during prolonged hospitalization. This bacterium causes frequent infections due to its natural adaptability and abundance, and it has emerged as the most important species with which to analyze multidrug resistance. Here, we isolated multidrug-resistant strains from clinical specimens derived from patients with nosocomial infections, then compared the susceptibility of these strains to commercial synthetic antibiotics and to ethanoic acids prepared from organic apple cider, sugar molasses, dates, grapes, and grains using conventional standard microbiological techniques. *Pseudomonas aeruginosa* ATCC 27853 was the standard control strain. Because ethanoic acids contain carboxylic acids that control the formation of *P. aeruginosa* biofilm, they should help to suppress infection with this bacterium.

MATERIALS AND METHODS

All chemicals, reagents, and media components for this study were purchased from Sd Fine Chem Ltd, (Kolkata, India) Loba Chemie Pvt., Ltd. (Mumbai, India), HiMedia (Mumbai, India), bioMérieux SA., (Marcy l’Étoile, France or Sigma Aldrich Corp. (St. Louis MO, USA).

Isolation and purification of *P. aeruginosa*

Green pus, green catheters, urine, greenish ventilators, abscesses, throat swabs, nasal swabs, aspirates and collected from patients with nosocomial infections, and samples collected at surgery were processed using standard aseptic microbiological techniques. The samples were streaked onto plates containing cetrimide agar, a selective and differential medium, and incubated overnight at 37°C to allow *P. aeruginosa* to secrete exopigments. The isolates were identified and purified based on positive oxidase tests. The authenticity of the standard *P. aeruginosa* ATCC 27853 strain was confirmed in the same manner.

Antimicrobial susceptibility tests

The antimicrobial susceptibility of the clinical isolates and the standard strain to standard synthetic chemical antibiotics was assessed using rapid e-tests. Briefly, the isolates were inoculated onto separate plates containing Mueller-Hinton agar and standard e-test plastic strips were infused with each antibiotic and incubated with the isolates at 37°C overnight to develop zones and ellipses. The interaction of the ellipse was taken as the minimum inhibitory concentration (MIC), whereas the zone indicated the susceptibility of the antibiotic to the bacterium. Conventional standard was employed to observe The susceptibility of the *P. aeruginosa* isolates and the standard strain to five organic ethanoic acids was assessed by the standard diffusion method using Kirby-Bauer discs. Briefly, the isolates were inoculated separately on Mueller-Hinton agar and standard e-test plastic plates with the infused discs for 24 h at 37°C to form zones, indicating the sensitivity of the bacterium toward the organic ethanoic acids. The MIC and minimum bactericidal concentration (MBC) reflecting the ability of ethanoic acid to kill the bacterium were estimated using the standard tube dilution method. Briefly, the isolates were separately inoculated into ethanoic acids diluted in peptone water, and incubated for 24 h at 37°C. The absence of turbidity indicated the sensitivity of the bacterium to the test agent. The last dilution with turbidity determined the MIC value of the test agent against the bacterium. The MBC was determined by inoculating each dilution of MIC onto separate agar plates.
for each isolate\textsuperscript{5,10,11,12}, then incubating them for 24 h at 37°C. The first dilution with no growth defined the MBC of the acid toward the bacterium \textit{Pseudomonas aeruginosa} ATCC278.

**RESULTS AND DISCUSSION**

The results obtained from the e-tests of the susceptibility of \textit{P. aeruginosa} to standard synthetic chemical antibiotics compared with organic ethanoic acid showed that none of the clinical \textit{P. aeruginosa} isolates were resistant to organic ethanoic acid, whereas all the clinical isolates were resistant all the standard synthetic antibiotics tested. The best and worst antibiotic zone diameters for the standard synthetic chemical antibiotics against the clinical isolates

### Table 1. Comparison of \textit{Pseudomonas aeruginosa} susceptibility to standard antibiotics according to e-test zone diameters (mm).

| Specimen          | Antibiotics | AK   | PM   | TZ   | TX   | GM   | MP   | NI   | PTC  | TC   | TLC  | TM   | TS   | S   | I   | R   |
|-------------------|-------------|------|------|------|------|------|------|------|------|------|------|------|------|-----|-----|-----|
| Green pus         |             | 2 R  | 3 R  | 2 R  | 4 R  | 2 R  | 20 S | 18 S | 2 R  | 19 S | 21 S | 12 I | 20 S | 5   | 1   | 6   |
| Green cath        |             | 13 I | 2 R  | 3 R  | 2 R  | 4 R  | 12 I | 20 S | 22 S | 20 S | 20 S | 25 S | 20 S | 20 S | 6   | 2   | 4   |
| Urine             |             | 4 R  | 3 R  | 2 R  | 4 R  | 2 R  | 20 S | 20 S | 20 S | 20 S | 20 S | 20 S | 20 S | 10 I | 5   | 2   | 5   |
| GV                |             | 5 R  | 2 R  | 2 R  | 4 R  | 2 R  | 10 I | 20 S | 21 S | 21 S | 20 S | 20 S | 20 S | 10 I | 5   | 3   | 4   |
| Abscess           |             | 4 R  | 3 R  | 2 R  | 4 R  | 2 R  | 20 S | 20 S | 10 S | 10 S | 21 S | 20 S | 20 S | 20 S | 5   | 2   | 5   |
| Throat swabs      |             | 2 R  | 2 R  | 4 R  | 2 R  | 20 S | 20 S | 20 S | 10 I | 21 S | 20 S | 20 S | 20 S | 20 S | 20 S | 20 S | 20 S | 20 S |
| Aspirates         |             | 2 R  | 3 R  | 2 R  | 4 R  | 2 R  | 4 R  | 2 R  | 9 I  | 11 I | 21 S | 20 S | 20 S | 20 S | 4 R  | 2   | 2   | 8   |
| Wound             |             | 4 R  | 2 R  | 4 R  | 2 R  | 9 I  | 11 I | 6 R  | 20 S | 20 S | 20 S | 20 S | 20 S | 20 S | 20 S | 20 S |
| Nasal swabs       |             | 2 R  | 22 S | 20 S | 10 I | 20 S | 20 S | 20 S | 20 S | 20 S | 20 S | 20 S | 20 S | 10 I | 8   | 1   | 3   |
| Surgical sources  |             | 4 R  | 3 R  | 2 R  | 4 R  | 2 R  | 4 R  | 2 R  | 9 I  | 11 I | 21 S | 20 S | 20 S | 20 S | 3   | 2   | 7   |

**Control**

| Antibiotics | AK | PM | TZ | TX | GM | MP | NI | PTC | TC | TLC | TM | TS |
|-------------|----|----|----|----|----|----|----|-----|----|-----|----|----|
| Green pus   | 2 R| 3 R| 2 R| 4 R| 2 R| 20 S| 18 S| 2 R | 19 S| 21 S| 12 I| 20 S|
| Green cath  | 13 I| 2 R| 3 R| 2 R| 4 R| 12 I| 20 S| 22 S| 20 S| 25 S| 21 S| 20 S|
| Urine       | 4 R| 3 R| 2 R| 4 R| 2 R| 20 S| 20 S| 20 S| 20 S| 20 S| 20 S| 20 S|
| GV          | 5 R| 2 R| 2 R| 4 R| 2 R| 10 I| 20 S| 21 S| 21 S| 20 S| 20 S| 20 S|
| Abscess     | 4 R| 3 R| 2 R| 4 R| 2 R| 20 S| 20 S| 10 S| 10 S| 21 S| 20 S| 20 S|
| Throat swabs| 2 R| 2 R| 4 R| 2 R| 20 S| 20 S| 20 S| 10 I| 21 S| 20 S| 20 S| 20 S|
| Aspirates   | 2 R| 3 R| 2 R| 4 R| 2 R| 4 R| 2 R| 9 I | 11 I| 21 S| 20 S| 20 S|
| Wound       | 4 R| 2 R| 4 R| 2 R| 9 I | 11 I| 6 R | 20 S| 20 S| 20 S| 20 S| 20 S|
| Nasal swabs | 2 R| 22 S| 20 S| 10 I| 20 S| 20 S| 20 S| 20 S| 20 S| 20 S| 20 S| 20 S|
| Surgical    | 4 R| 3 R| 2 R| 4 R| 2 R| 4 R| 2 R| 9 I | 11 I| 21 S| 20 S| 20 S|
| ATCC 27853  | 0.25| 0.25| 0.25| 0.25| 0.25| 0.25| 0.25| 0.25| 0.25| 0.25| 0.25| 0.25|

**Table 2. Comparison of \textit{Pseudomonas aeruginosa} susceptibility to standard antibiotics determined as minimum inhibitory concentrations in e-tests.**

| Specimen          | Antibiotic MIC (µg/mL) | AK | PM | TZ | TX | GM | MP | NI | PTC | TC | TLC | TM | TS |
|-------------------|------------------------|----|----|----|----|----|----|----|-----|----|-----|----|----|
| Green pus         |                        | 1  | 1  | 0.25| 0.25| 0.5 | 0.5| 0.5 | 0.25| 0.25| 0.5 | 0.5|
| Green cath        |                        | 1.25| 0.5| 0.25| 0.5 | 0.5 | 0.5| 0.5 | 0.25| 0.25| 0.25| 0.5|
| Urine             |                        | 1  | 1  | 0.25| 0.25| 0.5 | 1  | 1  | 1   | 0.5 | 0.25| 0.5|
| GV                |                        | 1  | 1  | 0.25| 0.25| 0.5 | 0.5 | 0.5 | 0.25| 0.25| 0.25| 0.5|
| Abscess           |                        | 1  | 1  | 0.25| 0.25| 0.5 | 1  | 1  | 1   | 0.25| 0.25| 0.25|
| Throat swabs      |                        | 0.25| 0.25| 0.25| 0.25| 0.5 | 1  | 1  | 0.25| 0.25| 0.25| 0.25|
| Aspirates         |                        | 0.25| 0.5| 0.25| 0.25| 0.5 | 0.5 | 0.5 | 0.25| 0.25| 0.25| 0.5|
| Wound             |                        | 0.25| 0.5| 0.25| 0.25| 0.5 | 0.5 | 0.5 | 0.25| 0.25| 0.25| 0.5|
| Nasal swabs       |                        | 0.25| 0.5| 0.25| 0.25| 0.5 | 0.5 | 0.5 | 0.25| 0.25| 0.25| 0.5|
| Surgical          |                        | 0.25| 0.5| 0.25| 0.25| 0.5 | 0.5 | 0.5 | 0.25| 0.25| 0.25| 0.5|
| ATCC 27853        |                        | 0.25| 0.5| 0.25| 0.25| 0.5 | 0.5 | 0.5 | 0.25| 0.25| 0.25| 0.5|
| Mean MIC*         |                        | 0.75| 0.625| 0.625| 0.625| 0.625| 0.625| 0.625| 0.625| 0.375| 0.375| 0.375|

I, intermediate susceptibility; R, resistant; S, susceptible.

*For all samples. Cath, catheter; GV, greenish ventilator; MIC, minimum inhibitory concentrations (µg/mL); Surgical, sourced during surgery.
of *P. aeruginosa* were 23 and 2 mm, respectively, compared with 29 and 20 mm, respectively, for organic ethanoic acids. Among the 10 clinical isolates, the strain from the wound sample was resistant to 8 of the 12 standard synthetic chemical antibiotics, and sensitive to piperacillin/tazobactam (PTC) and tetracycline (TC) with a zone diameter of 20 mm. This strain also had intermediate sensitivity toward gentamicin (GM) and meropenem (MP) with zone diameters of 9 and 11 mm, respectively. The clinical *P. aeruginosa* isolates from the wound sample were the most multidrug-resistant among the strains isolated herein, with different degrees of sensitivity to all tested antimicrobial agents. These results showed that organic ethanoic acids were more effective than standard synthetic antibiotics even against the most multidrug-resistant of the clinical *P. aeruginosa* isolates obtained herein.

Tables 1 and 3 show detailed comparison of the antimicrobial susceptibilities of all *P. aeruginosa* isolates from patients with nosocomial infections to standard synthetic chemical antibiotics and organic ethanoic acids. The mean MIC of standard antibiotic dilutions in e-tests and of organic ethanoic acids ranged from 0.375 – 0.750 µL/mL and 0.25 – 0.875 µL/mL, respectively for all *P. aeruginosa* isolates tested. Tables 2 and 4 respectively show details of the MIC of the standard synthetic chemical antibiotics and organic ethanoic acids for all *Pseudomonas aeruginosa* isolates from patients with nosocomial infections. The mean values of the MIC of organic ethanoic acid ranged from 0.5 – 1.25 µL/mL toward all isolates, and showed that organic ethanoic acids were more effective than standard synthetic chemical antibiotics for all *P. aeruginosa* isolates tested.

Table 3. Comparison of diffusion zone diameters (mm) among *Pseudomonas aeruginosa* isolates that were susceptible to organic ethanoic acids in conventional assays.

| Sample              | Apple cider | Sugar molasses | Dates | Grapes | Grains |
|---------------------|-------------|----------------|-------|--------|--------|
| Green pus           | 22          | 22             | 22    | 22     | 22     |
| Green catheter      | 20          | 22             | 19    | 22     | 22     |
| Urine               | 21          | 21             | 20    | 22     | 24     |
| Greenish ventilator | 22          | 22             | 23    | 22     | 23     |
| Abscess             | 27          | 22             | 24    | 22     | 24     |
| Throat swab         | 24          | 22             | 23    | 26     | 26     |
| Aspirates           | 23          | 26             | 24    | 22     | 22     |
| Wound               | 24          | 22             | 22    | 22     | 22     |
| Nasal swab          | 22          | 22             | 22    | 22     | 22     |
| Surgical samples    | 25          | 22             | 22    | 22     | 22     |
| ATCC 27853 (Control)| 29          | 26             | 26    | 21     | 20     |
| Mean Zone diameter (mm) | 24.5       | 24             | 21.5  | 23.5   | 23     |

Table 4. Comparison of minimum inhibitory concentrations of organic ethanoic acids among *Pseudomonas aeruginosa* isolates.

| Sample             | Organic ethanoic acids (µg/mL) |
|--------------------|--------------------------------|
|                    | Apple cider | Sugar molasses | Dates | Grapes | Grains |
| Green pus          | 0.25        | 0.5            | 1     | 1      | 1      |
| Green catheter     | 0.25        | 0.25           | 1     | 1      | 0.75   |
| Urine              | 0.25        | 0.5            | 1     | 0.75   | 1      |
| Greenish ventilator| 0.25        | 0.25           | 0.5   | 0.5    | 1      |
| Abscess            | 0.25        | 0.5            | 0.75  | 0.5    | 0.75   |
| Throat swabs       | 0.25        | 0.25           | 0.5   | 0.5    | 1      |
| Aspirates          | 0.25        | 0.75           | 0.75  | 1      | 0.5    |
| Wounds             | 0.25        | 0.75           | 0.75  | 0.75   | 0.5    |
| Nasal swab         | 0.25        | 0.25           | 1     | 0.5    | 1      |
| Surgical sources   | 0.25        | 0.75           | 1.25  | 1      | 0.5    |
| ATCC 27853         | 0.25        | 0.25           | 0.5   | 0.5    | 0.5    |
| Mean MIC (µg/mL)*  | 0.25        | 0.5            | 0.875 | 0.75   | 0.75   |

MIC, minimum inhibitory concentrations. *Derived from all samples.
Fig. 1. Comparison of mean e-test susceptibility of 10 clinical Pseudomonas aeruginosa isolates to standard synthetic chemical antibiotics.

Fig. 2. Comparison of mean conventional susceptibility of 10 clinical Pseudomonas aeruginosa isolates to organic ethanoic acids derived from different sources.

Table 5. Comparison of minimum inhibitory concentrations of organic ethanoic acids among Pseudomonas aeruginosa isolates determined by conventional assays.

| Sample         | Organic ethanoic acids (µg/mL) |
|----------------|-------------------------------|
|                | Apple cider | Sugar molasses | Dates | Grapes | Grains |
| Green pus      | 0.5         | 0.75           | 1.25  | 1.25   | 1.25   |
| Green catheter | 0.5         | 0.5            | 1.25  | 1.25   | 1.25   |
| Urine          | 0.5         | 0.75           | 1.25  | 1.25   | 1.25   |
| Greenish ventilator | 0.5    | 0.5            | 1.25  | 0.75   | 1.25   |
| Abscess        | 0.5         | 0.75           | 1.25  | 0.75   | 1.25   |
| Throat swabs   | 0.5         | 0.5            | 0.75  | 0.75   | 1.25   |
| Aspirates      | 0.5         | 0.5            | 1.25  | 1.25   | 0.75   |
| Wound          | 0.5         | 0.5            | 1.25  | 0.75   | 1.25   |
| Nasal swabs    | 0.5         | 0.5            | 1.25  | 0.75   | 0.75   |
| Surgical       | 0.5         | 0.1            | 1.5   | 1.25   | 0.75   |
| ATCC 27853     | 0.5         | 0.5            | 1.25  | 1.25   | 1      |
| Mean MIC (µg/mL) | 0.5    | 0.75           | 1.25  | 1.25   | 1      |

*For all samples. MIC, minimum inhibitory concentrations.

P. aeruginosa isolates. Table 5 shows a detailed comparison of the MBC of organic ethanoic acids for all the P. aeruginosa isolates.

The comparative findings of the antimicrobial susceptibility test values for the 10 clinical isolates of P. aeruginosa derived from patients with nosocomial infections suggests that organic ethanoic acid is significantly more effective than standard synthetic antibiotics.

CONCLUSIONS

The most important finding of the study was that the ATCC 27853 standard strain of P. aeruginosa was more susceptible to organic ethanoic acids than to standard synthetic antibiotics.

The susceptibility of the 10 clinical isolates of P. aeruginosa derived from the patients was variable compared with that to organic ethanoic acids. This finding indicates that the multidrug-resistant strains in the clinical isolates might be the result of excessive antibiotic administration, especially during prolonged hospitalization. Multidrug-resistant strains emerge due to constant disregard for warnings about antibiotic use published by the World Health Organization (WHO). The present findings showed that natural remedies such as organic ethanoic acids can serve as an alternative to synthetic antibiotics not only for eradicating multidrug-resistant organisms but also for other infections, which together with zero side effects could help save many lives.
ABBREVIATIONS

AK, amikacin; ATCC 27853- Standard Pseudomonas aeruginosa strain; GM, gentamicin; I, intermediate; MP, meropenem; NI, nitrofurantoin; PM, cefepime; PTC, piperacillin/ tazobactam; R, resistant; S, sensitive; SZD, susceptibility zone diameter (mm); TC, tetracycline; TLC, ticarcillin/ clavulanic acid; TM, tobramycin; TS, trimethoprim; TX, ceftriaxone; TZ, ceftazidime.

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AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript and/or the Supplementary Files.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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