*International Journal of Sub-Saharan African Research (IJSSAR)*
*Vol. 4, Issue 2, pp. 266-275, June 2026, ISSN: 3043-4467 (Online), 3043-4459 (Print)*
**https://doi:10.5281/zenodo.21066522**

# Fusidic Acid as an Alternative Therapeutic Option for *Staphylococcus aureus* Infections in the Era of Rising Antimicrobial Resistance

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## ABSTRACT

**Background:** Fusidic acid is one of the most effective antibiotics against methicillin resistant *Staphylococcus aureus* (MRSA), making it a valuable option for the treatment of *S. aureus* infections. The present study aimed at evaluation of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of tested virulent *S. aureus* strain CM05 on fusidic acid
**Objective:** This study examined actions of Fusidic Acid as an alternative therapeutic option for *Staphylococcus aureus* Infections in the era of increased antimicrobial resistance.
**Methodology:** Clinical Microbiology strain (CMO5) of *S. aureus* from Dr Sue Langs' collection with her approval and Oxford strain of *S. aureus* for MIC control were obtained and reconfirmed before use in this study between April to December, 2014. Repetition of the experiment on the isolates were carried out through cultural, gram reaction, catalase, agglutination and API web tests. The confirmed isolates were subcultured into Blood agar (BA) (Colombia agar), Brain heart infusion agar (BHI), Muller-Hinton broth & agar (MH) and Trypton soya broth (TSB). All media were from Oxoid, Ltd, Basingstoke Hampshire, and England. Antimicrobial susceptibility testing was performed according to CLSI, 2014 guidelines.
**Results:** The strain showed hemolysis on blood agar, round shaped and size of 2 µm. Gram reaction showed positive cocci in cluster, catalase and coagulase positive. API identification revealed a 97.8% probability of the isolate being *Staphylococcus aureus* with biochemical reactions used to obtain 7 unique numbers that was matched with API web software based on colour. The MIC and MBC of fusidic acid against strain CM05 were 0.12 µg/mL and 0.5 µg/mL, respectively in relation to oxford strain of 0.12- 0.5 µg/mL range that was used as control.
**Conclusion:** The work showed Fusdic acid as an alternative in antibiotic of choice in treating *S. aureus* related infection and confirmed the identity of our strain for this study which is CM05.
**Unique Contribution:** This study has offered an alternative means for rising antimicrobial resistance.
**Key Recommendation:** Further study in DNA, RNA, and cDNA of CMO5 strain with the experiment to be performed more than twice for appropriate results comparison and bridge of information gap in the research area.

**Keywords:** *Staphylococcus aureus* strain CM05, antimicrobial susceptibility, Fusidic acid.

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## INTRODUCTION

The name *Staphylococcus aureus*, first *Staphylococcus aureus* was first described in the nineteenth century, originated from the latin word '*aurum'* for gold, and '*albus'* meaning white (Otto, 2014; Strauß et al., 2017). Fusidic acid is a steroidal antibiotic belonging to the fusidane class and exhibits potent activity primarily against Gram-positive bacteria, particularly *Staphylococcus* species. (Falagas, Grammatikos, & Michalopoulos, 2008; Kang et al., 2025). Fusidic acid is a bacteriostatic antibiotic commonly applied topically in its ointments form and as eye drops but may also be administered as tablets or infusion. The global rise in antimicrobial resistance has renewed interest in the clinical use of fusidic acid (Falagas et al., 2008; González-López et al., 2025).

The mechanism of action is based on bacterial protein synthesis inhibition. It is active mainly on gram-positive bacteria like *S. species*. It stops translocation throughout protein synthesis and prevents the accumulation of ppGpp (Agarwal, Kumar, Tyagi, & De, 2014). The drug was acknowledged for use as sodium fusidate, in Europe and other countries. The continued clinical relevance of fusidic acid is largely attributable to its activity against MRSA (Fernandes, 2016).

Fusidic acid has effects beyond skin infections and active in the management of people with prosthetic joint-related bone infections (Ray, Singh, & Gupta, 2019). Fusidic acid is not administered alone to treat *S. aureus* infections at reduced dosage. It may be likely to use it as monotherapy when used at greater doses (Vassiliou, Demetriades, & Scott, 2002). Topical fusidic acid has long been used in Europe, often in combination with another antibiotic such as mupirocin or systemic agents, particularly for skin infections like impetigo. This practice is supported by European guidelines and clinical experience, though resistance concerns have led to more cautious use (Alsulami et al., 2025; Xie, Li, Yang, & Dong, 2025). The combination of fusidic acid with other antibiotics also helps to prevent resistance that would occur when administered alone and increase synergistic effect of the antibiotic.

Mechanisms underlying resistance to fusidic acid are explained in the changes that occur in the elongation factor G, which appear as natural mutant and are kept at reduced amount in an ideal population of *S. aureus* of $10^6$ to $10^8$ Cfu/ml (Hajikhani et al., 2021; Nhan, Leclercq, & Cattoir, 2011). Resistance to this antibiotic is not usually seen except in the case of cross infections commonly in a hospital setting (Abdelmassih, Ismail, Kashef, & Essam, 2024; Alsulami et al., 2025; Wilkinson, 1998).

Despite the established clinical usefulness of fusidic acid against *S. aureus*, limited information exists regarding its activity against virulent strain CM05. Determining the susceptibility profile of this strain may provide useful baseline information for future molecular and antimicrobial resistance studies. Therefore, this study evaluated the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of fusidic acid against *S. aureus* strain CM05.

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## MATERIALS AND METHODS

### *Isolate characterization and subculture*

This laboratory-based experimental study was conducted between April and December 2014 at the Department of Clinical Microbiology, Glasgow Caledonian University, United Kingdom.
Clinically confirmed strains of *S. aureus* – Clinical Microbiology strain (CMO5) of *S. aureus* from Dr Sue Langs' collection and Oxford strain of *S. aureus* for MIC control were obtained and reconfirmed before use in this study following ethical approval obtained from the appropriate institutional review board for reuse. The isolates were confirmed through gram reaction, catalase, agglutination and API web tests. The confirmed isolates were subcultured into Blood agar (BA) (Colombia agar), Brain heart infusion agar (BHI), Muller-Hinton broth & agar (MH) and Trypton soya broth (TSB). All media were from Oxoid, Ltd, Basingstoke Hampshire, England.

### *Growth Curve/Viable Count:*

The strains (CMO5 and Oxford) were streaked onto BA and incubated at 37°C overnight. Oxford strain served as control during MIC confirmation. Colonial morphology was observed after overnight incubation. Overnight broth cultures were set up by picking single colony onto 2x 10ml MH sterile broth and further incubated overnight at 37°C with periodic shaking. Two (2) ml of the overnight broth culture of CMO5 was transferred onto a 250 ml flask containing 100 ml of MH sterile broth placed in a rotating water bath where 1000 µl was taken every 30 minutes starting from 0 minutes and Optical Density (OD) 600nm was measured for growth curve. 100 µl was also taken from the same flask simultaneously to Phosphate Buffer Saline (PBS) and 4-fold (4) serial dilution was made starting from $10^2$. Last three dilutions were plated on BHI agar in duplicate by pour plate technique until 3.50 hours. From 4 hours to 6hours, the serial dilutions increased to 6-folds (6 x) and the last four dilutions ($10^3$,$10^4$,$10^5$ and $10^6$) were each plated out in duplicate on to BHI agar and incubated at 37°C overnight. The viable count was recorded, and plates were stored in the cold room at 4°C for subsequent sub-culturing.

### *Preparation of antibiotic (Fusidic acid) stock solution:*

Fusidic acid with potency of 980 µg/mg, used in this study, was obtained from Sigma (Poole, Dorset, UK) and prepared according to CLSI, 2014 guideline.
The initial weight of Fusidic acid used was calculated from the formula:

$$\frac{1000 \times V \times C}{P} = W$$

Where P =potency of the antibiotic (980 µg/mg), V= volume require in ml, C= final concentration of solution in mg/1000 and W= weight of the antibiotic in mg. Thus, the initial weight of Fusidic acid used was 1000/980x10x10 =102.0mg.
Stock solution A of 10,000mg/L was prepared and 500 µ/l was pipetted into a sterile universal bottle with 4.5ml of distilled water to make stock solution B and from Stock solution B, 100 µ/l was pipetted to 9.9ml of distilled water to make stock C while 100 µ/l of C was added to 9.9ml distilled water for stock solution D and this was repeated from D to prepare stock solution E to suit the antibiotic dilution ranges as represented in table 1.

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**Table 1: stock solutions Preparation**

| Sterile Universal bottle | Final antibiotic concentration (µ/l) | Volume of stock solution (µ/l) | Stock solution | Volume of diluent (ml) |
|---|---|---|---|---|
| 1 | 16 | 320 | A | 9.9 |
| 2 | 8 | 160 | B | 9.8 |
| 3 | 4 | 80 | B | 9.9 |
| 4 | 2 | 40 | B | 9.9 |
| 5 | 1 | 200 | C | 9.9 |
| 6 | 0.5 | 100 | C | 9.8 |
| 7 | 0.25 | 50 | C | 9.9 |
| 8 | 0.12 | 120 | D | 10 |
| 9 | 0.06 | 60 | D | 9.9 |
| 10 | 0.03 | 30 | D | 10 |
| 11 | 0.015 | 150 | E | 9.9 |

***Setting up of Microtiter plates:***
After the stock preparation, 96 well plates were prepared using 75 µl of broth without antibiotic to serve as a control and 75 µl of the antibiotic (Fusidic acid) from 0.015-16 was added into the labelled wells in triplicate. Each of the micro titer plates was covered with sticky lid before storage to avoid evaporation and was stored in the freezer to conserve the potency of the antibiotic.

***Setting up of overnight broth culture of CM05 and Oxford strain:***
Two sterile universal bottles with 10 ml BHI broth were inoculated with single colony from the subculture of the respective strain and the broth cultures were incubated at 37°C overnight with periodic shaking.

***Minimum Inhibitory Concentration (MIC):***
This is defined as the lowest concentration of antimicrobial that will inhibit all or almost all the growth of an organism after sub-culture onto antibiotic free media (McConnell, 2006; Rodríguez-Melcón, Alonso-Calleja, García-Fernández, Carballo, & Capita, 2021; Wiegand, Hilpert, & Hancock, 2008). After overnight incubation of broth culture, one of the three 96 well plate containing 75 µl of the antibiotic was defrost on ice and an equal volume of the broth culture (75 µl) was added after the overnight culture was diluted to OD 0.3(600nm) to give $10^8$cfu/ml. A further 1 in 100 dilutions gave $10^6$cfu/ml and 75 µl were inoculated into each well. BHI broth was used as control. The microtitre plate was incubated overnight at 37°C after which the MIC was read to 0.12 for CMO5 and 0.28 for oxford strain respectively.

***Minimum Bactericidal Concentration (MBC):***
The MBC is the lowest concentration of the antibiotic that will kill a bacterial strain. To carry out MBC, the individual microtitre well showing no growth (clear wells) was picked and inoculated onto the quartered plates of MH agar, incubated overnight at 37°C and the result was recorded.

***Time Kill Curve:***
Two flasks containing 100ml of MH broth was placed in the water bath where 500µl of the overnight broth culture of CMO5 was added into each of them from 0.3 OD 108cfu/ml at 600nm

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followed by 1:100 dilution, 106cfu/ml. The amount of antibiotic (stock C) required to be added into one of the flasks was estimated and obtained from stock A. 100µl of the prepared MIC was added into one of the flasks.1000 µl was taken every 30 minutes from both flasks for optical density measurement starting from 0 minute followed by 100µl which was diluted in 6-folds and $10^2$, $10^3$ and $10^4$ plated out in duplicate for flask with antibiotic and no antibiotic by pour plate method onto BHI agar. Measurement of the OD was continued until 0.1 was reached, and the series dilutions was increased to 7-folds. $10^4$, $10^5$, $10^6$ and $10^7$ and was plated out in duplicate for flask without antibiotic while $10^3$, $10^4$, $10^5$ and $10^6$ was plated for flask with antibiotic using the same method up to 6.30 minutes. The culture plates were incubated overnight at 37°C.

Two BA plates were used to set up purity of Oxford strain and CMO5 followed by incubation in the same manner as above. To ensure that the antibiotic preparation for time kill curve was correctly prepared, 150 µl 16XMIC of Fusidic acid, 0.12 was added into the first columns of microtitre plate and 75 µl transferred to the first two rows containing 75 µl of CMO5 and another 2 rows preceding that with 75 µl Oxford strain while 75 µl MH broth was added into the third 2 rows to serve as negative control and was incubated for 18 hours at 37◦C. After the overnight incubation, MIC plate was checked followed by viable count of plates with and without antibiotic, and purity plating to rule out contamination.

**RESULT**

In this study, Clinical Microbiology strain (CMO5) of *S. aureus* from Dr Sue Langs' collection was characterised for identity and effects of Fusidic acid. The strain showed hemolysis on blood agar, round shaped and size of 2µm. Gram reaction showed positive cocci in cluster, catalase and coagulase positive.

Further confirmation with API analysis identified the isolate as *Staphylococcus aureus* with an identification probability of 97.8% (fig. 1). Fig. 2 showed biochemical reactions used to obtain 7 unique numbers that was matched with API web software based on color.

Fig. 3 showed the CMO5 grown in MH broth for 6 hours. At 0 time, the absorbance was 0.1 at 600nm. Lag phase lasted for 2 hours before it grew exponentially for 5hrs and then entered stationary phase.

MIC and MBC result were 0.125 µg/mL and 0.55 µg/mL in relation to oxford strain of 0.12- 0.55 µg/mL range that was used as control and exhibited bacteriostatic nature with Fusidic acid as shown in time kill curve (fig. 4a & b).

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| GOOD IDENTIFICATION | |
|---|---|
| Strip | API STAPH V4.1 |
| Profile | 6736153 |
| Note | POSSIBILITY OF Staph intermedius IF OF VETERINARY ORIGIN |

| Significant taxa | %ID | T | Tests against | |
|---|---|---|---|---|
| Staphylococcus aureus | 97.8 | 1.0 | | |

| Next taxon | %ID | T | Tests against | |
|---|---|---|---|---|
| Staphylococcus simulans | 1.0 | 0.74 | MAL 11% | |

| Complementary test(s) | YELLOW | dTURANOSE | |
|---|---|---|---|
| Staphylococcus aureus | +(-) | +(-) | |
| Staphylococcus intermedius | - | - | |

**Fig. 1: API web software identification ( Sterlin bioline, London UK)**

**Fig. 2. Analytical Profile Index (API)**

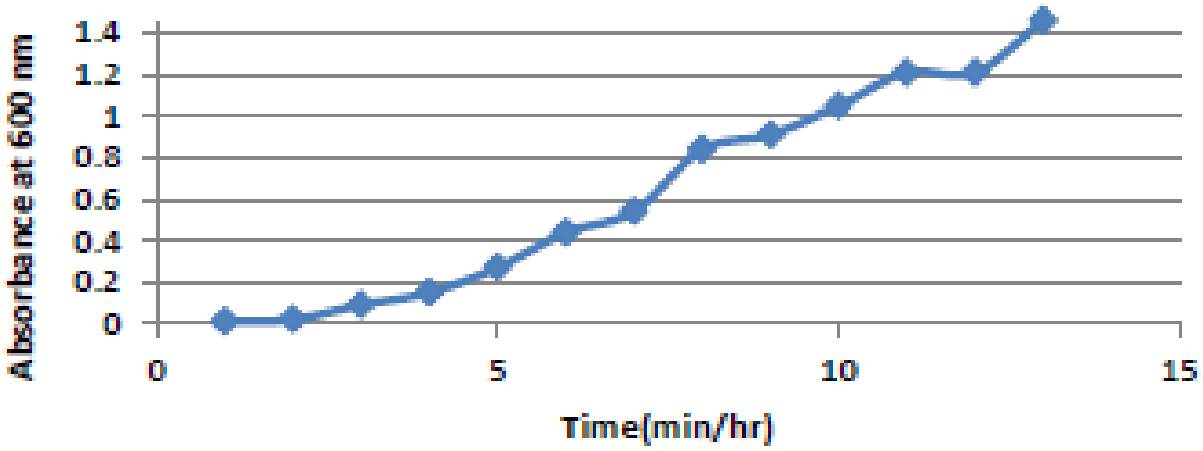

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**Fig. 3: Graph of Absorbance of CMO5 at 600nm Vs Time (min/hr)**

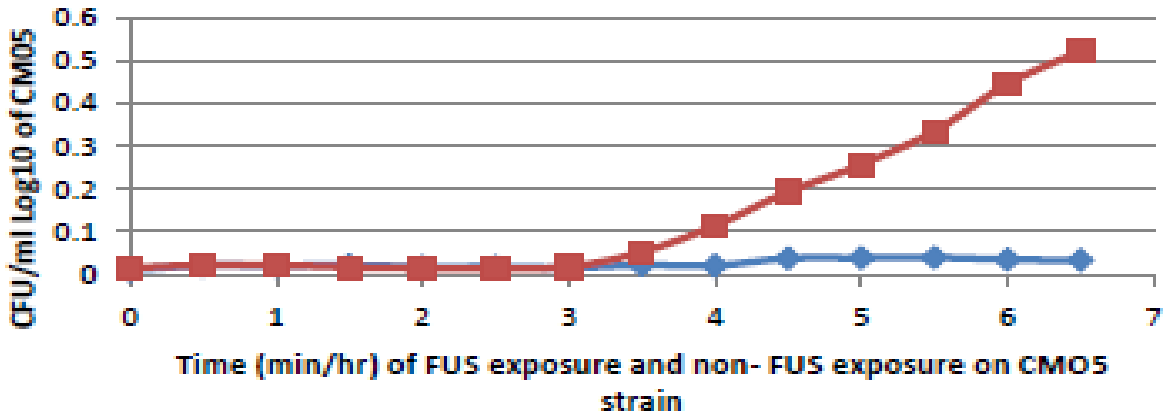


**Figure 4a. Time-kill curve showing changes in viable counts (log10 CFU/mL) of CM05 following exposure to fusidic acid compared with untreated control.**

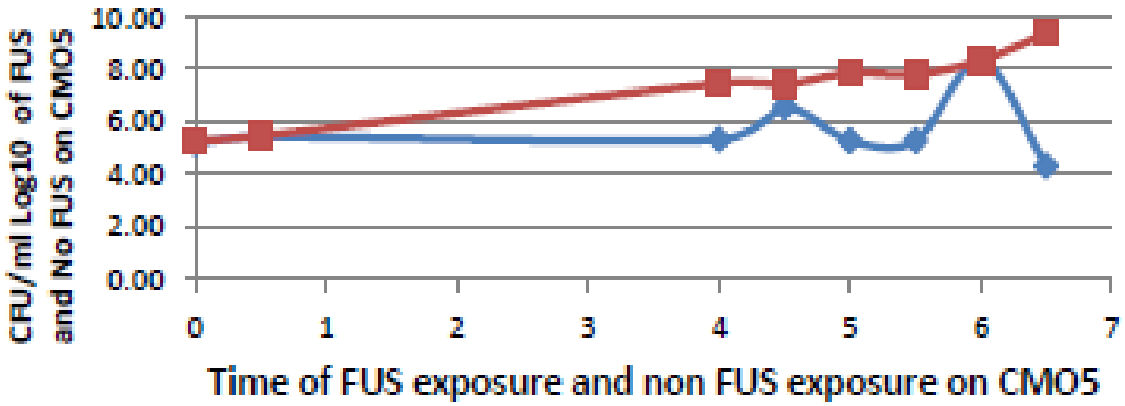


**Figure 4b. Comparative growth kinetics of CM05 in the presence and absence of fusidic acid over time**

The orange color in the graph of fig. 4 shows CMO5 without the effect of antibiotic while the blue line below it shows the bacteriostatic activity.

## DISCUSSION AND CONCLUSION

CMO5 *S. aureus* strain and Oxford strain used in this study was confirmed to have morphological (using results from BA plate reading and gram stain reaction) and biochemical (with catalase, agglutination for coagulase/protein A and API web software) characteristics consistent with S. aureus based on the features described by Otto (2010).

When the strain was examined for bacterial growth phases using MH broth for 6 hours with time starting from zero time, growth started immediately with lag phase for 2 hours using 600nm OD before log phase which lasted for 5 hours at 600nm OD and was found to have followed the usual bacterial growth rate up to stationary phase according to Presscott (2010) but decline phase

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followed a different trend of further increase and this may have been due to stress and other yet to be uncovered factors which led to further bacterial multiplication/growth as represented in fig. 2. API web software was also used to confirm the identity of the CMO5 to *S. aureus* and very high percentage was recorded.

The behaviour of CMO5 with Fusidic acid in the determination of minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) agreed with documented study elsewhere by Wiegand et al. (2008). Fusidic acid exhibited bacteriostatic effect on CM05 as can be seen in fig. 4(a) with the blue line going horizontal while the orange line which represented CMO5 with no antibiotic effect as control rose and growth continued exponentially. Fig. 4 (b) was also plotted using different variables and same trend of bacteriostatic effect was deduced but the curve did not work perfectly due to some unknown factors to be investigated in future works.

This project agreed with documented records about *S. aureus* and as shown in the introduction, but contrary results were obtained in bacterial growth phases where the strain refused to enter decline bacterial growth but instead, entered another exponential growth which may have resulted due to stress or other factors which further studies will uncover.

The results of Fusidic acid concentration above MIC agreed with the postulation of other authors such as Alsulami et al. (2025), Frosini et al. (2017) and González-López *et al*. (2025) McConnell, (2006) and Flagas et. al (2008) which stated that increased or combination of the antibiotic such as Vancomycin or Rifampicin could be used to treat cases of *S. aureus* resistant cases like MRSA and others.

In conclusion, characterisation of CMO5 strain culturally, biochemically and confirmed it to be 97.8% *S. aureus* and showed bacteriostatic effect when treated with Fusdic acid. The use Whole-genome sequencing, resistance gene profiling, and transcriptomic analyses are recommended to further characterize CM05 and elucidate mechanisms of fusidic acid susceptibility.

**Ethical clearance**
Ethical consent was sought and obtained in this study and explanation was given to show that the use of the isolates was purely for academic purposes.

**Acknowledgements**
I acknowledge Dr Sue Lang Ph D students for assisting me with data collection. I equally appreciate the Glasgow University Library staff for their cooperation and support.

**Sources of funding**
The study was funded by TET Fund Nigeria through Ebonyi State University Abakaliki

**Conflict of Interest**
There was no conflict of interest

**Authors' Contributions**
The whole work was done by me.

**Artificial Intelligence (AI) Use Disclosure**
No generative Artificial Intelligence (AI) technologies were used in the writing, analysis, or preparation of this manuscript.

**Data availability statement**
The datasets on which conclusions were made for this study are available on reasonable request.

**Citation**
Azi, S. O. (2026). Fusidic Acid as an Alternative Therapeutic Option for *Staphylococcus aureus* Infections in the Era of Rising Antimicrobial Resistance. *International Journal of Sub-Saharan African Research,* 4(2), 266-276

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