RESEARCH ARTICLE

Mursamacin: a novel class of antibiotics from soil-dwelling roundworms of Central Kenya that inhibits methicillin-resistant *Staphylococcus aureus* [version 1; peer review: 2 approved, 1 approved with reservations]

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Abstract

Antibiotic-resistant bacteria, also called “superbugs”, can at worst retrogress modern medicine to an era where even sore throats resulted in death. A solution is the development of novel types of antibiotics from untapped natural sources. Yet, no new class of antibiotic has been developed in clinical medicine in the last 30 years. Here, bacteria from insect-killing *Steinernema* roundworms in the soils of Central Kenya were isolated and subjected to specific molecular identification. These were then assayed for production of antibiotic compounds with potential to treat methicillin-resistant *Staphylococcus aureus* infections. The bacteria were identified as *Xenorhabdus griffinae* and produced cell free supernatants that inhibited *S. aureus*. Fermenting the bacteria for 4 days yielded a heat stable anti-staphylococcal class of compounds that at low concentrations also inhibited methicillin-resistant *S. aureus*. This class contained two major compounds whose identity remains unknown. Thus *X. griffinae* isolated from *Steinernema* roundworms in Kenya have antimicrobial potential and may herald novel and newly sourced potential medicines for treatment of the world's most prevalent antibiotic resistant bacteria.

Keywords

Antibiotic resistant bacteria, *Xenorhabdus* bacteria, novel antimicrobials

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Introduction

Antibiotic resistant bacteria, otherwise known as “superbugs”, are an imminent threat to every existing healthcare system as they could obviate current clinical antibiotics and thereby retrogress humanity to that dark age of lethal sore throats¹. Of note is methicillin-resistant *Staphylococcus aureus* (MRSA). In this study, we examine the antimicrobial activity of *Xenorhabdus griffinae* fermentation media against MRSA.

MRSA not only causes human diseases such as mastitis, chronic open wound infections and endocarditis, but also livestock diseases such as mastitis in dairy cattle and lameness in poultry and rabbits², that together result in economic losses of billions of dollars to the agricultural sector³. In both humans and animals, an MRSA infection can quickly turn lethal. This is because MRSA is resistant to two antibiotic classes, beta lactams and macrolides, and only lipopeptides and glycopeptides remain effective⁴; this development has contributed in large part to the rise of the vancomycin-resistant *S. aureus* superbugs. A recommended solution¹ to the superbug conundrum is to develop novel classes of antibiotics that can replace those to which disease-causing bacteria have mutated to resist their inhibitory effect.

A potential source of novel anti-MRSA antibiotics are *Xenorhabdus* bacteria⁵ that naturally dwell in the guts of *Steinernema* roundworms. These 1 mm-long⁶ roundworms are found in soils worldwide⁷ and live by infecting and killing insects such as moths, caterpillars and weevils. The *Xenorhabdus* bacteria carried in their gut aid this insect killing lifestyle. Explicitly, *Steinernema* roundworms enter an insect body and release *Xenorhabdus* bacteria that secrete insecticidal toxins that quickly kill the insect⁸. To secure this rich food source for the roundworms only, the *Xenorhabdus* produce an armory of antibiotic compounds that effectively destroy competing soil fungi and microorganisms⁹, a mechanism that has been demonstrated as having medical potential against human diseases^{8,10–14}. Each *Xenorhabdus* species has been demonstrated to produce its own unique array of antibiotics¹¹ and this is prompting new studies on the classes of antimicrobial compounds produced. For example, *X. cabalinasi* JM26 from Jamaica led to the discovery of nemaucin, a novel and highly potent antibiotic compound against methicillin-resistant *S. aureus*¹⁵.

Politically, “superbugs” are today’s top global health issue exemplified by the 2016 United Nations High Level General Meeting’s agenda being antibiotic resistant bacteria: this is only the fourth time in its 80-year history that a health issue has been the reason for this annual meeting. Technically, superbugs have been detected in all 114 countries recently surveyed with the number of pan drug resistant superbugs, those immune to every antibiotic available, on the rise¹. Accordingly, Kenyan prevalence levels of MRSA have been steadily increasing^{16,17}.

Previously¹⁸ we demonstrated that *Xenorhabdus* bacteria from Kenya can produce antibiotics against MRSA. However, the identity of the species, the antibiotic classes it produces and their inhibitory concentrations remain unknown. Here, we elucidate the specific molecular identity of Kenyan *Xenorhabdus* isolates and determine the efficacy of produced antimicrobial compounds against MRSA. Our findings highlight a novel antibiotic class

designated “mursamacin”¹⁹ obtained from *Xenorhabdus* bacteria found in Kenyan soils, which is highly active against methicillin-resistant *S. aureus*.

Methods

Bacterial and nematode strains

MRSA strain 133 cultures were obtained as a gift from Dr. John Ndemi of the Kenya Medical Research Institute, Centre for Microbiology Research, Nairobi, Kenya. Pure nematode cultures of *Steinernema* roundworm isolates were obtained from the nematode culture collection of Horticulture Research Institute Thika, Kenya.

Using the indirect haemolymph method²⁰ *X. griffinae* strains XN45 and L67 were isolated from *Steinernema* sp. Scarpo and *Steinernema* sp. L67 nematodes respectively. The isolates were cultured on *Xenorhabdus* differential media NBTA, composed of nutrient agar (Himedia) supplemented with 0.0025% (w/v) bromothymol blue (Sigma-Aldrich) and 0.004% (w/v) 2,3,5 triphenyl tetrazolium chloride (Sigma-Aldrich)²⁰. Identification of the bacteria as *Xenorhabdus* was based on the presence of the following characteristics: swarming motility on NBTA of 1% (w/v) agar concentration; swimming motility on NBTA of 0.5% (w/v) agar concentration; and yellow green colony pigmentation on NBTA⁵. Specific identification of the bacteria was done by multi locus sequence typing of the *16s rRNA*, *serC* and *recA* genes (See [Dataset 1](#)).

Molecular methods

Total DNA extraction from the bacterial strains was done using FastDNA®SPIN Kit for Soil (MP Biomedicals, USA). Isolation of a 1397 base pair(bp) *16s rRNA* gene fragment was done by Polymerase Chain Reaction (PCR) with primer sequences (Inqaba Biotech) as follows: (27f-AGA GTT TGA TCA TGG CTC AG) and (1391r-ACG GGC GGT GTG TGC)²¹. The genes were amplified in a 25 µl reaction volume containing final concentrations of 0.5 U Q5 DNA polymerase (New England Biolabs, USA), 200µM each dNTP, 2mM MgCl₂ and 0.05µM of each primer. Cycling conditions were set at 98°C for 30 s, 40 cycles of 98°C for 30 s, 42°C for 15 s (first 20 cycles) and 47°C for 15 s (final 20 cycles), 72°C for 1 min and a final extension of 72°C for 2 min (MJ Research PTC-100, USA). Isolations of 670bp *serC* and 400bp *recA* gene fragments were done by PCR with primers (*recA*-FW CCA ATG GGC CGT ATT GTT GA) and (*recA*REV-TCA TAC GGA TCT GGT TGA TGA A) and (*serC*-FW CCA CCA GCA TAC TTG TCC TTT C) and (*serC*REV-AAA GAA GCA GAA AAA TAT TGC AC) respectively²². They were amplified in a 50 µl reaction volume containing final concentrations of 2 U MyTaq® DNA polymerase (Bioline, USA), 200µM of each dNTP, 3mM MgCl₂ and 0.4µM of each primer. For both, cycling conditions were set at 95°C for 1 min, then 40 cycles of 95°C for 15 s, 52°C for 15 s, 72°C for 40 s, with a final extension of 72°C for 5 min (Thermo Scientific Arktik, USA).

PCR products were visualized on 1.2% (w/v) agarose gels stained with ethidium bromide at final concentrations of 0.5 µg/ml. Typical electrophoresis conditions were 4V/cm for 72 min. Expected bands were excised and purified with Quick Clean II Gel extraction kits® (Genscript, USA). Products were outsourced for sequencing (Macrogen, Netherlands), and obtained sequences were quality checked, assembled and poor quality base calls trimmed in BioEdit²³ and MEGA6²⁴ software suites.

Phylogenetic analysis

Phylogenetic reconstruction was performed using a multi locus concatenate of *16s rRNA*, *serC* and *recA* genes sequences, that jointly constituted 2076 positions. A dataset of n=13 (1= from this study and 12= public databases) was used that contained the 11 strains of *Xenorhabdus* and a *Photorhabdus luminiscens* (the out-group sequence) that had public database sequences of all three genes (see data files). Database sequences were checked for quality and ambiguous nucleotides resolved in the MEGA6 software suite²⁴. Multiple sequence alignments were performed in the same suite using the MUSCLE algorithm²⁵. The evolutionary history was inferred by the ML method based on the Generated Time Reversible (GTR) Model (500 bootstraps) (Nei and Kumar 2000). Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The analysis involved 13 nucleotide sequences. All positions containing gaps and missing data were eliminated resulting in a total of 2076 positions used in the reconstruction. *Xenorhabdus* DNA sequences generated in this study were deposited in the DNA databank of Japan with the partial *16s rRNA* gene sequences of *X. griffinae* L671, L672, L673, L675, XN45 assigned the following accession numbers respectively; AB987698.1, AB987700.1, AB987701.1, AB987699.1, AB987697.1. The partial *recA*, *serC* gene sequences of *X. griffinae* L67 and XN45 were assigned LC096094, LC096092 and LC096093, LC096091 respectively. Percentage sequence similarities between gene sequences from this study and other *16s rRNA*, *recA* and *serC* gene database sequences was determined using blast searches²⁶.

Bacterial fermentation

Fermentation was done using *X. griffinae* XN45 bacterial cultures. Multiple colonies (2–3) of an individual isolate were selected, inoculated into 5 ml of Luria Bertani (LB) media containing 1% tryptone, 0.5% yeast extract and 1% NaCl (w/v) and incubated on a rotatory shaker at 150 rpm at 33°C for 24 h. These served as 1% (v/v) starter inocula. Sterile LB media (500 ml) was dispensed into sterile 1-L Erlenmeyer flasks, with starter cultures (5 ml) thereafter inoculated and LB media incubated at 150 rpm at 33°C for 355 h, 180 h and 108.5 h. LB with no inoculum was also incubated to serve as a control for sterility. After fermentation, cells were removed by centrifugation of broths at 20,000 g for 25 min at 4°C (Beckman Avanti J-25, USA) followed by decanting cell free supernatants (cfs). These were heat-treated by autoclaving at 121°C and 15 p.s.i for 20 min to yield a sterile heat stable fraction of the whole broth extract (antibiotic) that was designated “mursamacin”. These were stored at 4°C until use.

Broth macro dilution inhibition assay against MRSA

The broth macro dilution assay was prepared as previously described¹¹ with modifications, using antibiotics obtained from each of the fermentation durations (180.5 h and 355 h) as the antibiotic and MRSA as the test bacterium. MRSA overnight cultures were inoculated into each dilution, at a final concentration of 2.3×10^4 cfu/ml, and then incubated for 18 h at 37°C without agitation. The following controls were included in every

replicate: negative control of 2× LB media inoculated with bacteria without antibiotic; sterility control of 2× LB media with no inoculated bacteria; and sterility control of undiluted antibiotic with no inoculated bacteria.

After incubation, turbidity of each dilution was measured by determining the $A_{600\text{nm}}$ (See dataset files). This was used in the following formula, modified from Houard, Aumelas¹³ that included a correction factor for inhibition by the broth media to calculate the percentage growth inhibition of bacterial cultures by an antimicrobial.

$$\{g - g_x\} \times \frac{100}{g}$$

Where $g = A_{600\text{nm}}$ of bacteria in broth culture without antibiotic, and $g_x = A_{600\text{nm}}$ of bacteria in broth culture with antibiotic.

Fractionation of lyophilized powders of mursamacin

To extract an organic heat stable mursamacin class of antibiotics, cfs from a 108.5 h fermentation reaction were lyophilized to yield a yellow powder, whose measured amounts were dissolved in known volumes of methanol, and vortexed for 2 min to yield a dark orange methanol extract. These were centrifuged at 20,000 g for 13 min at room temperature to separate liquid methanol extracts from insoluble residues. Methanol extracts were pipetted into sterile 1.5 ml tubes while pelleted solids were aseptically air dried and then incubated at 37°C overnight to evaporate residual methanol. To provide a negative control, the same procedure was repeated for lyophilized powders of the fermentation media. The difference in weight of powder before and after methanol extraction was then measured to determine the concentration of total dissolved compounds in the methanol extracts.

Broth microdilution assay of organic heat stable mursamacin antibiotics against MRSA

This was modified from a previously described method²⁷. To determine the minimum inhibitory concentrations (MICs) against MRSA, 100 µl of known concentrations of organic heat-stable fractions of mursamacin antibiotics were dispensed into sterile 96-well micro-titre plate starting wells. These were left in a biological safety cabinet to evaporate methanol resulting in visible orange solid residues; each was then re-dissolved in 200 µl RPMI media supplemented with 5% (v/v) LB²⁸. Every other well along each row was filled with 100 µl RPMI media supplemented with 5% (v/v) LB; 100 µl of starting well mixture was then dispensed to the subsequent well resulting halving the antibiotic concentration. This was repeated for all wells along the rows resulting in a 2-fold dilution series. MRSA inocula (10 µl) were used that had been previously prepared from plate cultures dissolved in physiological saline to a turbidity of 0.5 Mcfarland standard (ca. concentration= 2.6×10^6 cfu/ml), then subjected to 10^{-3} dilution in RPMI media supplemented with 5% (v/v) LB. A positive control row of Daptomycin and negative control of methanol extract of LB media only was incorporated in every replicate. Plates were incubated at 37°C for 21 h without agitation. Experiment was performed in 10 replicates in three reproductions.

High performance liquid chromatographic analysis

To identify the compounds contained in the organic heat-stable fraction of mursamacin antibiotics, analytical reverse phase chromatography of the methanol extract was performed as previously described¹³ and modified to use of a c18 column (Agilent Zorbax Eclipse Plus C18; 3.5µm, 4.6 ×100 nm) under isocratic conditions of 60% acetonitrile and uv detection of 224 nm.

Results and discussion

Xenorhabdus griffinae strains are found in Kenya

The *Xenorhabdus* isolates identified were most closely related to *Xenorhabdus griffinae* (Figure 1). The apt thresholds for *Xenorhabdus* species identification based on sequence similarities are currently considered to be 98.65% and 97% for *16s-rRNA* and for both *recA* and *serC* gene fragments respectively^{29,30}. In this study, sequences similarities to *X. griffinae* strains, including type strains, were 99.52%, 98.57% and 97.68% for *16s rRNA*, *recA* and *SerC* respectively (See data files)

Xenorhabdus griffinae has only previously been isolated from Indonesia³¹, Malaysia³² and South Africa³³; therefore our data strongly suggest a new strain of *X. griffinae* originating from Central Kenya.

X. griffinae produces heat stable anti-staphylococcal compounds

The growth of methicillin-resistant *S. aureus* was inhibited when cultured in *X. griffinae* cell-free supernatants (cfs) that we termed 'mursamacin' (Figure 2). Furthermore, cfs were obtained by various *X. griffinae* fermentation durations and had the following percentage of growth inhibition against methicillin-resistant

S. aureus at neat concentrations: 81% (cfs from 180 h ferment), 94% (cfs from 355 h ferment) (Figure 3). In both instances, cfs were heat-sterilized by autoclaving and pH adjusted to that of the control (6.6–7.0), indicating that inhibition was due to heat stable compounds contained therein.

Previous studies^{9,11–13,34,35} have demonstrated that *Xenorhabdus* bacteria are prolific antimicrobial producers with each species producing its unique array of antibiotics that often contained novel compounds. Consistent with these reports, our data demonstrate unprecedented evidence of heat-stable *X. griffinae* antimicrobials and further suggests that their production is affected by how long *X. griffinae* is cultured in fermentation media.

An organic heat-stable fraction mursamacin antibiotics is highly effective against methicillin-resistant *S. aureus*

An organic heat-stable fraction of mursamacin antibiotics inhibited methicillin-resistant *Staphylococcus aureus* at a standardized concentration of 8.25 µg/ml while its negative control- organic extract of autoclaved fermentation media only- displayed growth at all concentrations confirming this inhibition as due to antibiotic compounds only (Table 1). However, this concentration was 17-fold higher than the positive control Daptomycin that gave 0.5 µg/ml.

Currently, there are two major clinical antibiotics with inhibitory concentrations against methicillin-resistant *S. aureus* that are considered effective: Daptomycin (0.5 µg/ml) and Vancomycin (2 µg/ml)⁴. On the other hand, the larger majority today's clinical drugs have inhibitory concentrations against methicillin-resistant *S. aureus* considered that are considered ineffective; Azithromycin

Figure 1. Phylogenetic tree of *Xenorhabdus* species based on the maximum likelihood method. The tree was based on a concatenate of *ss-rRNA*, *recA*, and *serC* gene sequences and was reconstructed with a general time reversal model and test of phylogeny of 500 bootstrap replicates. *X. griffinae* isolated from this study (red triangle) clustered in the *X. griffinae* clade (red), and was most closely related to *X. griffinae* from Malaysia.

Figure 2. Inhibition of methicillin-resistant *S. aureus* by mursamacin antibiotics. MRSA cultures were incubated with (tube a) and without (tube b) mursamacin antibiotics respectively. A clear tube denotes no bacterial growth while turbid tube denotes bacterial growth.

Figure 3. Growth inhibition of methicillin-resistant *S. aureus* by heat stable fractions of mursamacin antibiotics produced by varying fermentation durations. The longer fermentation duration (355 h) produced antibiotics that were generally more inhibitory to methicillin-resistant *S. aureus*. Dotted line graphs represent linear equations derived from the raw inhibition values. The high R-squared values demonstrate that the concentration of the antibiotic was predominantly responsible (97–98%) for the level of percentage growth inhibition.

(128 µg/ml), Amoxicillin/Clavulanic acid (64 µg/ml), Ceftriaxone (64 µg/ml) and Erythromycin (32 µg/ml) and Imipenem (16 µg/ml)⁴. Yet our data demonstrate a heat stable class of compounds with an inhibitory concentration of 8.25 µg/ml. This strongly suggests that antimicrobials contained in this class are even more potent against methicillin-resistant *S. aureus*, as pure compounds.

Further high performance liquid chromatographic analysis revealed that this fraction contained two major compounds, eluted at 1.862 min and 2.775 min and absorbed at 224_{nm}

when dissolved in methanol (Figure 4). Previous studies have characterized classes of organic *Xenorhabdus* antibiotics that were highly effective against *S. aureus*^{11,36} and had peaks absorption ranges from 200–230_{nm}¹⁵. Of note, the PAX lipopeptides¹⁰ isolated *X. cabanillasii* and *X. nematophila* were highly effective against MRSA at concentrations of 0.5 µg/ml. Yet in contrast to our results, no class has been characterized as heat stable and isolated from *X. griffinae*.

In conclusion, we demonstrate that *X. griffinae* antibiotic compounds termed “mursamacin” contained an organic heat stable

Table 1. MICs of mursamacin against methicillin-resistant *S. aureus*.

Antibiotic	MIC (µg/ml) against MRSA			Average MICs	Standardized MICs*
Mursamacin	59.83 ^a	32.34 ^b	28.36 ^c	40.18	8.5
Positive Control (Daptomycin)	2.08	2.5	2.5	2.36	0.5
Negative Control (Solvent Extract of fermentation media)	>92.38	>46.19	>72.13	>70.23	>14.88

MICs of a,b,c are averages of 3,4,3 replicates respectively (See data files).

*These are projected MICs values should tests be done under standard assay conditions.

Figure 4. HPLC Chromatographs of organic mursamacin antibiotics. Top chromatograph represents the solvent only while bottom chromatograph is of solvent containing organic mursamacin antibiotics. Two dominant compounds, indicated by the red arrows, were detected in this fraction.

fraction are highly effective against methicillin-resistant *S. aureus* and antimicrobial activity seems to be attributed to two dominant uncharacterized compounds. This may offer a founding stone for further development of clinical drugs from *X. griffinae*, giving hope to thousands of patients affected by methicillin-resistant *S. aureus* infections.

Dataset 1. Raw data of antibiotics from soil dwelling roundworms of Central Kenya inhibiting methicillin resistant *Staphylococcus aureus*

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All raw data supporting the study are provided.

Data availability

F1000Research: Dataset 1. Raw data of antibiotics from soil dwelling roundworms of Central Kenya inhibiting methicillin resistant *Staphylococcus aureus*, [10.5256/f1000research.9652.d136966](https://doi.org/10.5256/f1000research.9652.d136966)³⁷

Accession numbers of sequences generated from this study are: AB987698.1, AB987700.1, AB987701.1, AB987699.1, AB987697.1, LC096094, LC096092 and LC096093, LC096091.

Author contributions

RMA, PNN, LNN conceived the study and carried out the research. NA designed the experiments and supervised the study. RMA wrote the manuscript. All authors were involved in the revision of the manuscript and have agreed to the final content.

Competing interests

No competing interests were disclosed.

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Khushbu Sharma

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Introduction

1. 3rd Para, Line 9 "The *Xenorhabdus* produce an armory of antibiotic compounds...."
Can author define some names of antibiotic compounds produced by *Xenorhabdus* spp.
2. Last Para, Line last 3rd "designated "mursamacin"19 obtained from *Xenorhabdus* bacteria"
Name the spp. of *Xenorhabdus* bacteria used.

Methods

1. Page 3, Phylogenetic analysis, line 12, (Nei and Kumar 2000), according to reference style name should not be included despite use number and this reference is cited in text but not mentioned in Reference part of the manuscript.
2. Page 3, Bacterial fermentation, line 1, only one bacterial strain was used for fermentation what about the second strain mentioned in the manuscript?
3. Page 3, Bacterial fermentation, line last 4th, "These were heat-treated by autoclaving....."
If these were further autoclaved, even after inoculation, the antibiotics produced might get degraded. Explain.
4. Page 3, Bacterial fermentation, line 5th, The ideal temperature for growth of *Xenorhabdus* spp. is 28°C, then why 33°C had been used for bacterial fermentation?
5. Page 4, High performance liquid chromatographic analysis, line 1, "To identify the compounds....."
How can compounds be identified using HPLC only? This part is not explained well as per scientific standard.
6. Page 4, High performance liquid chromatographic analysis, line 5, "Eclipse Plus C18; 3.5um, 4.6 x100 nm...."

Instead of nm it should be mm and C-18 should be written like this not as author wrote.

7. How MIC's were calculated?

If my questions would be answered with full justification, I would give my full approval for this manuscript.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 03 November 2016

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Selcuk Hazir

Department of Biology, Faculty of Arts and Science, Adnan Menderes University, Aydin, Turkey

1. Mueller-Hinton media should have been used in antimicrobial tests instead of Luria Bertoni (LB).
2. The optimum growth temperature of *Xenorhabdus* spp. is 28°C. Why authors used 33°C for bacterial fermentation?
3. If autoclaved supernatant will be used, why centrifugation and filtration methods were used to remove the cells?
4. Not only autoclaved but also non-autoclaved cell-free supernatants should have been tested against *S. aureus* bacteria as well.
5. Tube-A in Figure-2 looks like there are several tubes in a beaker! This photo needs to be renewed.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 31 October 2016

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Habil Andras Fodor

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I consider the publication of Awori RM, Ng'ang'a PN, Nyongesa LN and Amugune NO, entitled as "[Mursamacin: a novel class of antibiotics from soil-dwelling roundworms of Central Kenya that inhibits methicillin-resistant *Staphylococcus aureus*](#)" is as follows as a well conducted research. I have read this submission.

I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard. I accept it but some little missing information should be added to get my approval (see details). The subject of paper is extremely important, since the "super bugs", that is the dramatically increasing numbers of multiresistant pathogens is a real danger for mankind and pose new challenge to the antibiotics researcher, and the research concept outline in this publication is excellent.

As for the title: it is appropriate for the content of the article.

The abstract represents a suitable summary of the work. As for the article content: the design, methods and analysis of the results from the study has been properly explained and they are appropriate for the topic being studied.

The information for the conclusions: the conclusions are sensible, well-balanced and justified on the basis of the results of the study. Enough information has been provided to be able to replicate the experiment. The data are presented in a usable format and all the data we need to understand has been provided.

Some detail:

- The paper reports the discovery of a new, heat stable antimicrobial (probably peptide) complex effective against the "superbug" MRSA in both in vitro tests and in larger scale. It is a very important discovery, even if we are still rather far to get a commercial product. The conception and the applied methods are suitable, and the discovery and proper molecular identification of the antibiotic producing *Xenorhabdus griffinae* from Kenya is very fortunate and great.
- I think that the quality of the research and the article is scientifically sound.

I have only one important question:

- Table 1: The reader need the definition how the "Standardized MIC" values were calculated from the "Average MICs". It seems to be a result of a statistical calculation, but I cannot be seen the source as mentioned.

I have two remarks concerning the content:

- Fig 4 and the related text in “Methods” and “Results and discussions” chapters: Some details related to “high performance liquid chromatographic analysis” is badly missing, such as the specific name of the method, the data of column, the eluting media, and at least an approximated molecular size of the two active fraction 1,862 and 2775.

If my question got an answer and my remark were taken into consideration, I would give my full Approval.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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