Antimicrobial Activities of Unconventional Compounds against Some Bacteria Associated with Skin Infections in Humans, Sheep and Goats

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Abstract: Bacterial skin infections are a common problem encountered in clinical practice and causing great economic losses for sheep and goats producers. Increasing multidrug resistance of pathogens paves the way for reconsidering alternative medicine. The present study was carried out to explore the antibacterial activities of different volumes; 5, 25, 50 and 100 µL of gold nanoparticles (NPS) and the aqueous and ethanolic extracts of garlic, turmeric and cinnamon at different concentrations (20, 40, 80 and 100%) against molecularly confirmed Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa and Escherichia coli isolated from skin pyogenic lesions in humans, sheep and goats using disc diffusion assay. The results compared with ciprofloxacin (5 µg mL⁻¹). Gold nanoparticles (NPS) 100 µL were highly effective against Pseudomonas aeruginosa and Escherichia coli in comparing with ciprofloxacin (5 µg mL⁻¹). Garlic has shown better activity against Staphylococcus aureus and Streptococcus pyogenes in aqueous solution while the ethanolic extract of cinnamon and turmeric was more efficient than the aqueous extract. Among the three tested spices, turmeric was the least effective against tested bacteria. The proven activity of 100 µL Gold nanoparticles (NPS) and 100% aqueous garlic extract compared with ciprofloxacin (5 µg mL⁻¹), suggests their use in clinical trials as an alternative medicine to reduce the side effects and progressively increasing drug resistance of pathogens.

Keywords: S. aureus, S. pyogenes, Ps. aeruginosa, E. coli, GNPS, Spices

Introduction

Bacterial skin infections are very common and they can range from merely annoying to deadly. Most bacterial infections of the skin are caused by gram-positive bacteria (Streptococcus, Staphylococcus) and gram-negative (e.g., Klebsiella, Escherichia coli, Pseudomonas) (AFHSC, 2013). Pyogenic skin infections of sheep and goats are of worldwide distribution, especially in developing countries. It causes severe economic losses to sheep and goats producers due to decrease live animal sales, condemnation and downgrading of carcasses and skin in abattoirs as well as the reduction in wool growth (Koutinas et al., 2007). Bacterial skin infections are treated with antibiotics, but there is a concern that widespread antibiotic use might lead to antibiotic resistance. Antibiotics resistance are an increasing public health problem. Multiple drug resistance has been developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. In addition, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression, allergic reactions. Certain strains of bacteria are able to produce substances which block the action of antibiotics or change their target or ability to penetrate cells (Aly, 2013). This situation forced scientists to search for a new treatment that does not generate resistance and present a good bactericidal property. Gold-nanoparticles have a great bactericidal effect on a wide range of bacteria; its bactericidal effect depends on the size and the shape of the particle (Nirmala et al., 2011). Nanoparticles can act as antibacterial and antifungal agents, due to their ability to interact with...
associated with pyogenic skin infections in comparing
Pseudomonas aeruginosa
Staphylococcus aureus, Streptococcus pyogenes,
extracts of garlic, cinnamon, turmeric against
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ciprofloxacin (5 µg mL
1)
different concentrations of the aqueous and ethanolic
find the inhibitory activity of gold nanoparticles and
pyogenic skin lesions in humans, sheep and goats and
different etiological pathogens which inhabit the
exudates of the lesions were collected from the affected
sample collection. A swab from the affected area or the
Munawarah, sterile cotton swabs which were moistened
Bacterial Isolates
Materials and Methods

Bacterial Isolates
Swabs from pyogenic skin lesions were collected
from patients in King Fahd hospital at Al-Madinah Al-
Munawarah, sterile cotton swabs which were moistened
with sterile saline to prevent drying were used for
sample collection. A swab from the affected area or the
exudates of the lesions were collected from the affected
sheep and goats. The specimens were brought to the
laboratory in a sterile container within one hour after
the collection and processed immediately and
inoculated on Maconkey agar and blood agar plates for
isolating the pathogens. The inoculated plates were
incubated at 37°C overnight. After incubation, the plates
were observed for growth and the isolated colonies were
identified by morphological and biochemical
characteristics. The tests performed include Gram
staining, Motility, Catalase, Oxidase, Indole, Methyl
Red, Voges-Proskauer, Citrate Utilization production,
Urease production, Triple Sugar Iron, Mannitol,
Phenylalanine (Koneman et al., 2005). The pure
cultures were refreshed on Nutrient agar slant and kept
in the incubator for 24 h at 37°C then stored at 4°C.

Molecular Identification of Isolates

Template DNA Preparation

DNA templates for PCR were obtained from
overnight bacterial cultures that were suspended in
200 mL of sterile distilled water and boiled for 15 min
(Usein et al., 2009).

Polymerase Chain Reaction (PCR)

Detection of a species-specific gene (uidA) in E. coli,
(ecfX) in Pseudomonas aeruginosa and 16S rRNA in
staphylococcus aureus and streptococcus pyogenes were
performed by conventional PCR (Moyo et al., 2007; Al-
Talib et al., 2009; Clifford et al., 2012). Primer
sequences and PCR conditions used for the study are
listed in Table 1. PCR was performed in the Takara
thermal cycler (Bio-Rad). PCR products were separated
and visualized by gel electrophoresis in 1.5% agarose
(Wako, Japan) in Tris-Acetate-Edta (TAE) buffer at 100
V. A 100 or 500 bp DNA ladder (one-step ladder, Wako)
was included in each agarose run, according to the
amplified product.

Gold Nanoparticles Preparation

Gold colloids 5, 25, 50 and 100 µL were prepared by
citrate thermal reduction method (Yang et al.,
2005). In this method, a gold solution was prepared by
adding 1 mL of 1% hydrogen tetra-chloroauric
(HAuCl4.3H2O) aqueous solution and 1.5 mL of 38.8
mM sodium citrate aqueous solution into 90 mL
boiling water. After the solution had turned purple-red
within the 30s, the solution was cooled quickly in an
ice bath. This indicated the dispersion of gold
particles with an average diameter of around 12 mm
width and 40 mm length. 0.2 mL of 0.1 M freshly
prepared acetyl Trimethyl Ammonium Bromide
(CTAB) aqueous solution was added to 20 mL of
prepared gold colloid at room temperature. Finally, 1
mL of 0.5 mM of 11-Mercaptoundecanoic (MUA)
aqueous solution was added to the gold colloid
modified by 0.1 mM of CTAB in order to restrain the
over much aggregation process.
Extract and Preparation of Plant Materials

The spices including Garlic (*Allium sativum*), Turmeric (*Curcuma longa*) and Cinnamon (*Cinnamomum zeylanicum*) were purchased from local market. The species were washed with distilled water thoroughly. Garlic (100 mg each) were washed by distilled water, homogenized using sterile mortar or blender and then saved through double layer of sterilized line mesh cloth to make 100% extract. Turmeric, Cinnamon (100 mg each) were washed by distilled water, homogenized using sterile mortar or blender and then saved through double layer of sterilized line mesh cloth to get the fine powder. Powdered spices were soaked in 200 mL of sterile distilled water and kept at room temperature for 24 h, then were filtered using Whatman filter paper. The filtrate was kept at room temperature until dry and thick layer formed. The drying thick layer was considered as 100% extract. This extract was stored at 4°C, further diluted to make different concentrations such as 80, 40 and 20% by mixing with appropriate volumes of distilled water. The ethanolic extract was prepared following the same procedure with the exception of solvent which was 95% ethanolic instead of sterilized distilled water.

Antimicrobial Activity Testing

Standard well agar diffusion method was carried out to detect the activity of gold nanoparticles and garlic, cinnamon, turmeric in aqueous and ethanolic extract against pathogenic bacterial isolates according to (Cheesbrough, 2000). Only 100 mL of the overnight 0.5 McFarland suspension of each isolate were inoculated into 100 mL warm nutrient agar medium (45-55°C). The media were poured in sterile plates and left for solidification. Each plate is called a seeded plate. The seeded plates with the isolated bacteria were cut by sterile cork borer to make holes (8 mm in diameter). Each hole was saturated (100 mL) with different volumes of gold nanoparticles (5, 25, 50 and 100 µL), 100 mL of the ethanol extract and aqueous extract of garlic, cinnamon and turmeric were transferred into each hole at all dilutions and 50 µL ciprofloxacin (5 µL) under aseptic conditions. Then, the plates were kept in the refrigerator for 2 h before incubation to permit diffusion of the extract before the growth of the tested isolates takes place. The plates were incubated at 37°C for 24 h and then examined for antibacterial activity. Duplicate plates were used for each isolate. The detection of clear inhibition zones around the wells on the inoculated plates is an indication of antimicrobial activity.

### Antimicrobial Activity of Ciprofloxacin (5 µg mL⁻¹)

Antimicrobial susceptibility testing of the isolates to ciprofloxacin (5 µg mL⁻¹) was performed using the standard disc diffusion method; using commercially available antimicrobial susceptibility discs (Kirby-Bauer SN DISC, Nissui Pharmaceuticals, Tokyo, Japan) according to Clinical and Laboratory Standards Institute (CLSI) instructions (Wikler, 2006).

### Results

**Bacterial Isolates**

Molecularly confirmed *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Escherichia coli* were obtained from pyogenic skin lesions.

**Characterization of Gold Nanoparticles**

Photo (1) shows TEM image of the obtained gold nanoparticles. The prepared gold nanoparticles were almost rod shape and separated from each other. The particle size mainly was 12 nm width and 40 nm length. The absorption spectrum of these gold rods has two characteristic absorption bands, one at 523 nm for transverse surface Plasmon resonance and the other at 753 nm for the longitudinal surface Plasmon band (Fig. 1).

### Antimicrobial Activity of Gold Nanoparticles

Antibacterial activities of gold nanoparticles increased with higher volume; 100 > 50 > 25> 5 µL. Gold nanoparticles (vol. 100 µL) showed great antimicrobial activities with the best inhibition zone against *Ps. aeruginosa* (27 mm), *E. coli* (25.5 mm) and *S. pyogens* (25 mm) and *S. aureus* (24 mm) as shown in Fig. 2 and Table 2. The effect of ciprofloxacin antibiotics was also studied, the best results were against *S. aureus* with an inhibition zone of (25 mm).

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**Table 1. PCR primers and conditions for amplification of virulence genes**

| Target gene | Primer designation | Primer sequence (5’-3’) | Length (bp) | PCR conditions * | PCR product | Reference |
|-------------|--------------------|-------------------------|-------------|-----------------|-------------|-----------|
| UidA (E. coli) | UidA-F | CAAAAGGCAGAGCAAGAT | 95°C, 30 s; 57°C, 30 s; 72°C, 30 s | 623 | 42 |
| | UidA-R | GCACAGACATCAAAAGAG |
| 16S rRNA (S. aureus) | 16S rRNA-F | GCAGCGGTATCCGAGATT | 95°C, 30 s; 57°C, 30 s; 72°C, 30 s | 597 | 43 |
| | 16S rRNA-R | CTATGATGGCAACTAAAG |
| ecIX (Ps. aeruginosa) | ecIXRT-F | AGGTTCGCTGTCGACAAAT | 95°C, 52°C, 30 s; 72°C, 30 s | 81 | 44 |
| | ecIXRT-R | TCCACCATGCTCGAGGAGAT |
| 16S rRNA (S. pyogens) | 16S rRNA-F | CAGTTCGATTGTAGGCTGC | 95°C, 30 s; 52°C, 30 s; 72°C, 30 s | 194 | The current study |
| | 16S rRNA-R | ACCCCAATCATCTATCCACC |

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Fig. 1. TEM image of gold nanoparticles

Fig. 2. Absorption spectra of gold nanoparticles

Fig. 3. Antibacterial activity of different volumes of gold nanoparticles against bacterial isolates compared with Ciprofloxacin (5 µg mL$^{-1}$)
Antimicrobial Activity of Garlic, Cinnamon and Turmeric Solution

Among the three tested spices, garlic has shown the best activity at all concentrations both in aqueous and ethanolic solution. Garlic has shown better activity against *S. aureus* (28 mm) in aqueous solution as compared to the other tested bacteria. Aqueous extract of garlic was more effective as compared to ethanolic extract. The activity of 100% garlic extract was comparatively more than of ciprofloxacin (5 µg mL⁻¹) (Fig. 3 and Table 3). The cinnamon ethanolic (100%) extract was also effective against *E. coli* (17 mm). The ethanolic extract of cinnamon was more efficient in its antibacterial activity as compared to the aqueous extract (Fig. 4 and Table 4). Turmeric was less effective against tested bacteria among the three tested spices. The ethanol extract of turmeric showed better results as compared to the aqueous ones showing the largest inhibition zone against *S. pyogenes* (14.1 mm) at 100% ethanolic extract (Fig. 5 and Table 5).

**Antimicrobial Activity of Ciprofloxacin (5 µg mL⁻¹)**

Ciprofloxacin showed high activity against the tested isolates, the largest inhibition zone (25 mm) was around *S. aureus*, followed by *S. pyogenes, E. coli, Ps. aeruginosa* (22, 20 and 20 mm respectively).

![Graph 4](image_url)

**Fig. 4.** Antibacterial activity of different concentrations of garlic against bacterial isolates compared with Ciprofloxacin (5 µg mL⁻¹)

![Graph 5](image_url)

**Fig. 5.** Antibacterial activity of different concentrations of cinnamon against bacterial isolates compared with Ciprofloxacin (5 µg mL⁻¹)
Table 2. Antibacterial activity of different volumes of gold nanoparticles against bacterial isolates compared with Ciprofloxacin (5 µg mL$^{-1}$)

| Bacterial isolates | Volume of gold nanoparticles (µL) | Ciprofloxacin (5µg mL$^{-1}$) |
|--------------------|----------------------------------|-------------------------------|
|                    | 5 µL                             | 25 µL                         | 50 µL                         | 100 µL                        |
| S. aureus          | 11 *                            | 16                            | 18                           | 20                           | 24                           | 25                           |
| S. pyogenes        | 11                              | 18                            | 20                           | 25                           | 22                           |
| E. coli            | 14                              | 22                            | 25                           | 25.5                          | 20                           |
| Ps. aeruginosa     | 10                              | 25                            | 26                           | 27                           | 20                           |

* : Mean value of inhibition zone (mm)

Table 3. Antibacterial activity of different concentrations of garlic against bacterial isolates compared with Ciprofloxacin (5 µg mL$^{-1}$)

| Bacterial isolates | Concentration | 20% aqueous ethanol | 40% aqueous ethanol | 80% aqueous ethanol | 100% aqueous ethanol | Ciprofloxacin (5 µg mL$^{-1}$) |
|--------------------|---------------|----------------------|---------------------|---------------------|----------------------|-------------------------------|
| S. aureus          | 11*           | 9.8                  | 16.0                | 11.0                | 20                   | 24                           | 25                           |
| S. pyogenes        | 10.5          | 9.0                  | 15.0                | 10.5                | 18                   | 15.0                         | 26                           | 21                           | 22                           |
| E. coli            | 10.0          | 9.0                  | 11.0                | 8.0                 | 9                    | 6.0                          | 25                           | 21                           | 22                           |
| Ps. aeruginosa     | 10.0          | 8.0                  | 11.7                | 9.0                 | 11                   | 7.5                          | 18                           | 15                           | 20                           |

* : Mean value of inhibition zone (mm)

Table 4. Antibacterial activity of different concentrations of cinnamon against bacterial isolates compared with Ciprofloxacin (5 µg mL$^{-1}$)

| Bacterial isolates | Concentration | 20% aqueous ethanol | 40% aqueous ethanol | 80% aqueous ethanol | 100% aqueous ethanol | Ciprofloxacin (5 µg mL$^{-1}$) |
|--------------------|---------------|----------------------|---------------------|---------------------|----------------------|-------------------------------|
| S. aureus          | 9*            | 8.6                  | 12.0                | 12.5                | 12.4                 | 15.0                         | 14.5                         | 16.0                         | 25                           |
| S. pyogenes        | 8             | 8.1                  | 12.1                | 12.5                | 13.0                 | 14.8                         | 14.6                         | 16.3                         | 22                           |
| E. coli            | 0             | 0.0                  | 9.3                 | 12.0                | 10.2                 | 14.8                         | 15.2                         | 17.0                         | 20                           |
| Ps. aeruginosa     | 0             | 0.0                  | 9.5                 | 11.0                | 11.3                 | 16.0                         | 14.9                         | 16.6                         | 20                           |

* : Mean value of inhibition zone (mm)

Table 5. Antibacterial activity of different concentrations of turmeric against bacterial isolates compared with Ciprofloxacin (5 µg mL$^{-1}$)

| Bacterial isolates | Concentration | 20% aqueous ethanol | 40% aqueous ethanol | 80% aqueous ethanol | 100% aqueous ethanol | Ciprofloxacin (5 µg mL$^{-1}$) |
|--------------------|---------------|----------------------|---------------------|---------------------|----------------------|-------------------------------|
| S. aureus          | 6.4*          | 8.0                  | 9.5                 | 10.5                | 10.1                 | 13.0                         | 11.6                         | 13.5                         | 25                           |
| S. pyogenes        | 7.0           | 9.9                  | 10.6                | 13.0                | 11.0                 | 13.5                         | 12.6                         | 14.1                         | 22                           |
| E. coli            | 8.0           | 8.8                  | 9.0                 | 9.5                 | 9.6                  | 10.3                         | 9.7                          | 10.5                         | 20                           |
| Ps. aeruginosa     | 8.3           | 8.5                  | 9.0                 | 9.3                 | 9.5                  | 10.1                         | 9.7                          | 10.4                         | 20                           |

* : Mean value of inhibition zone (mm)

Discussion

Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes and Pseudomonas aeruginosa are the most commonly isolated bacteria from pyogenic skin lesions (Lorrot et al., 2014; Zhang et al., 2014). The tested isolates in the current study were sensitive to ciprofloxacin, which is so far effective against the bacterial infections except in the case of the development of antimicrobial resistance (Sedláková et al., 2014). Antibiotic resistance poses a growing threat to health. Complementary and Alternative Medicine (CAM) therapies may provide a safer and a more effective treatment (MacKay, 2003). Antimicrobial activities of gold nanoparticles or plant extract differ according to the volume or concentration used and the tested isolates. The antimicrobial ability of gold nanoparticles might be referred to their size which is smaller than the bacterium and fungal cells (Nirmala et al., 2011). This makes them easier to adhere with the cell wall of the microorganisms.
E. coli

Garlic has better activity against activity of nanoparticles. Studies are required to know more about the biological induces death of the microorganism. However, further free radical quenching property; on the other hand, it interacts also with the building elements of the outer membrane and might cause structural changes; degradation and finally cell death. The antibacterial activities of the synthesized gold nanoparticles could be due to the susceptibility of pathogens cell wall and toxicity of metallic gold. Gold nanoparticles exert their antimicrobial action mainly changing the membrane potential and inhibiting ATP syntheses activities to decrease the ATP level, indicating a general decline in metabolism and it also inhibits the ribosome subunit for tRNA binding, indicating a collapse of a biological process. Gold nanoparticles also enhance chemotaxis in the early-phase reaction (Cui et al., 2012). Gold was tested by the well diffusion method against Microsporum gypseum (10 mm) and Trichophytorn rubrum (13 mm) (Karthik et al., 2013). The accumulation of positively charge gold nanoparticles (Au+) on the negatively charged cell membrane of microorganisms leads to conformational changes in the membrane, which loses permeability control which in turn cause the cell death (Chwalibog et al., 2010). This may be the possible mechanism of gold nanoparticles which Perform in vitro free radical quenching property; on the other hand, it induces death of the microorganism. However, further studies are required to know more about the biological activity of nanoparticles.

Garlic has shown the best activity and its aqueous extract was more effective as compared to ethanolic extract. Garlic has better activity against B. subtilis as compared to E. coli and aqueous extract of garlic was more effective as compared to ethanolic extract (Gull et al., 2012). Garlic was more effective against Gram-positive than Gram-negative bacteria (Srinivasans et al., 2009). The Gram-negative E. coli was comparatively resistant to Gram-positive bacteria, this may be due to the structure differences in cell membrane and cell wall structure, Gram-negative has outer membrane as well which further block the penetration of antibiotics including the extracts of spices making them resistant. The garlic extract is effective against different serotype of E. coli and also S. aureus (Naveed et al., 2013). Fresh garlic concentration 0.5-5.0% was sufficient to inhibit the growth of E. coli O157: H7 (Tessema et al., 2006).

The ethanolic extract of cinnamon was more efficient than its aqueous extract, as the antimicrobial component of the cinnamon bark is more soluble in ethanol as compared to water, but its activity was reported less as compared to the garlic (Naveed et al., 2013). Cinnamon extract possesses effective antibacterial properties against B. subtilis and E. coli, Gram-positive bacteria are more sensitive to essential oil of Cinnamon zeylanicum than Gram-negative bacteria (Buru et al., 2014). Some molecules of cinnamon oil (cinnamaldehyde and cinnamyl) bind to membrane proteins and inhibit peptidoglycan synthesis, the essential component of the bacterial cell wall, thereby increasing their antibacterial effect (Al-Mariri and Safi, 2014). The antimicrobial activity of cinnamon might be due to the presence of cinnamaldehyde compound which inhibits the amino acid decarboxylation activity in the cell which leads to energy deprivation and microbial cell wall (Ooi et al., 2006). These phenolic compounds are capable of further cellular destruction and inhibition by establishing the hydrophobic and hydrogen bonding of theses degradative phenolic compounds to membrane proteins resulting in portioning of the lipid bilayer (Juven et al., 1994). The antimicrobial activity at aqueous extracts could be due to anionic components such as thiocyanate, nitrate, chlorides and sulfates in addition to many other compounds naturally present in plants (Hill et al., 2014). The ethanolic extracts showed better results as compared to aqueous as being organic dissolves more organic compounds resulting in the release of a greater amount of active antimicrobial components (Cowan, 1999).

Turmeric extract was effective against all tested bacterial isolates in both aqueous and ethanolic solution; Turmeric was effective against E. coli. b. subtilis and s. aureus due to the presence of a phenolic compound, curcuminoid, the presences of essential oil, an alkaloid, curcuminis, turmerol and veleric acid are responsible for the antibacterial activity of turmeric (Moghadamtousi et al., 2014). Chloroform and isoamyl alcohol extracts of Cuminum cuminum showed a significant effect against Ps. aeruginosa, S. marcescnes and S. pyogenes (Awan et al., 2013). Aqueous and ethanolic extract of turmeric showed antibacterial activity against all bacterial cells isolated and tested at concentrations of 40, 80 and 100 %, but an aqueous and ethanolic extract of cinnamon has no effect on E. coli and Ps. aeruginosa at 20% concentration (Fig. 6). Curcumin inhibits bacterial cell division (Rai et al., 2008). Curcumin can be used safely in a wide range of concentrations without toxicity (Yeon et al., 2010).

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Fig. 6. Antibacterial activity of different concentrations of turmeric against bacterial isolates compared with Ciprofloxacin (5 µg mL$^{-1}$)

**Conclusion**

The present study concluded that gold nanoparticles and plant extract can be used either in aqueous or ethanolic solution to produce new therapeutics. Further research is required for possible future use of these extracts as alternatives to common antibiotics.

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**Author’s Contributions**

**Iman Shabana**: Contributed to the design and the establishment of experiments scheme. Sample collection, isolation and identification of the bacterial isolates. The performance of the disc diffusion assay. Data modeling, analysis and interpretation. The orientation of statistical graphics. Contributed to the writing of the manuscript. Important review contributions.

**Amira El-Adly**: Contributed to the design and the establishment of experiments scheme. Preparation of extracts and gold nanoparticles. The performance of the disc diffusion assay. Data modeling, analysis and interpretation. Contributed to the writing of the manuscript. Important review contributions.

**Ethics**

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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