12-1-2020

Octenidine/carbenicillin GUMBOS as potential treatment for oropharyngeal gonorrhoea

Kelsey M. Lopez
Louisiana State University

Jeffrey A. Hobden
LSU Health Sciences Center - New Orleans

Isiah M. Warner
Louisiana State University

Recommended Citation

Lopez, K., Hobden, J., & Warner, I. (2020). Octenidine/carbenicillin GUMBOS as potential treatment for oropharyngeal gonorrhoea. *Journal of Antimicrobial Chemotherapy, 75* (12), 3576-3581. https://doi.org/10.1093/jac/dkaa346

J Antimicrob Chemother 2020; **75**: 3576–3581
doi:10.1093/jac/dkaa346 Advance Access publication 24 August 2020

Journal of Antimicrobial Chemotherapy

Octenidine/carbenicillin GUMBOS as potential treatment for oropharyngeal gonorrhoea

Kelsey M. Lopez[1], Jeffrey A. Hobden[2] and Isiah M. Warner [1]*

[1]*Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803, USA;* [2]*Department of Microbiology, Immunology & Parasitology, LSU Health Sciences Center, New Orleans, LA 70112, USA*

*Corresponding author. E-mail: iwarner@lsu.edu

Received 13 March 2020; accepted 8 July 2020

Background: Reducing *Neisseria gonorrhoeae* colonies in the oropharynx is a viable solution to minimize the transmission of this bacterium amongst individuals.

Objectives: A strategy involving the electrostatic interaction between a common antiseptic and a discontinued antibiotic (i.e. octenidine and carbenicillin) was evaluated as a potential treatment for gonorrhoea. Octenidine/carbenicillin is a novel group of uniform materials based on organic salts (GUMBOS) with inherent *in vitro* antibacterial activity that comes from its parent antiseptic and antibacterial ions, octenidine and carbenicillin, respectively.

Methods: Antibacterial activities for octenidine dihydrochloride, disodium carbenicillin, octenidine/carbenicillin and stoichiometrically equivalent 1:1 octenidine dihydrochloride to disodium carbenicillin were assessed using the Kirby–Bauer disc diffusion assay for *N. gonorrhoeae* (ATCC 49226) and three clinical isolates. Predictive permeability using the Parallel Artificial Membrane Permeability Assay and cytotoxicity against HeLa cells was also evaluated.

Results: Additive *in vitro* antibacterial activities against *N. gonorrhoeae* were observed in this study, which suggests octenidine/carbenicillin could be a useful agent in reducing *N. gonorrhoeae* transmission and minimizing gonorrhoea infections. Octenidine/carbenicillin also exhibited bioequivalence to azithromycin and doxycycline, two currently prescribed antibiotics. Likewise, octenidine/carbenicillin had improved predicted permeability compared with octenidine dihydrochloride.

Conclusions: Antimicrobial GUMBOS synthesized in this study could be used as an adjunctive treatment approach to current drug therapies for oropharyngeal gonorrhoea infection control and prevention.

Introduction

The WHO estimates that 78 million new cases of gonorrhoea occur annually.[1] This is a pressing issue as *Neisseria gonorrhoeae*, the aetiological agent of the sexually transmitted disease gonorrhoea, has a long history of easily developing resistance to therapeutic regimens.[2] This study focuses on oropharyngeal gonorrhoea, as drug-resistant *N. gonorrhoeae* isolates from the oropharynx have emerged, most likely because there is poor drug penetration into pharyngeal tissue.[3] While current studies may have limitations, it has been recently demonstrated that gonococci could possibly be transmitted person to person strictly from 'deep kissing'.[4,5] *N. gonorrhoeae* can also persist in the oropharynx as most cases are asymptomatic or are misdiagnosed as other kinds of pharyngitis.[6,7] The oropharynx is thought to act as a 'silent reservoir', in which the gonococcus acquires its resistance determinants by horizontal gene transfer from commensal *Neisseria* species and other bacterial species in asymptomatic individuals. For this reason, decolonization of these individuals might prevent the emergence of MDR gonorrhoea.[8–10]

Currently, the recommended treatment is a dual regimen of ceftriaxone administered intramuscularly and azithromycin orally.[11] Although these antibiotics remain generally effective, resistance rates are rising globally. Antibiotic-resistant oropharyngeal gonorrhoea threatens to become a global crisis as conventional antibiotic therapy does not always reliably clear the pathogen from the throat. In 2010, a Swedish heterosexual man who presented with oropharyngeal gonorrhoea required several rounds of ceftriaxone with increasing dosage.[12] In 2017, a heterosexual man from the UK acquired drug-resistant oropharyngeal gonorrhoea in Southeast Asia. The infection did not respond to a high dosage of ceftriaxone (1 g) and spectinomycin, which indicates high-level resistance. The infection was cleared only after IV administration of ertapenem, an antibiotic

belonging to the potent carbapenem class of β-lactams that are often agents of 'last resort'.[13,14]

A possible solution for oropharyngeal gonorrhoea could be use of antiseptics, as they are unlikely to induce resistance. Antiseptics have been explored as treatments for acute gonorrhoea in the past[15] and antiseptic mouthwashes have been shown to have an antimicrobial effect on oropharyngeal gonorrhoea.[16] One such antiseptic, octenidine dihydrochloride, exhibits a broad spectrum of antimicrobial activity against Gram-negative and Gram-positive bacteria.[17] This antiseptic has shown significant efficacy in periodontology[18] and is even sold as a commercially available mouthwash under the name Octenisept®.[19] It has also been approved for use on skin, mucosal membranes and as wound antisepsis.[20] In this study, we aim to target and develop a strategy for minimizing gonorrhoea infections by developing an antiseptic-based compound that can be used as alternative therapy for gonorrhoea, specifically oropharyngeal gonorrhoea.

Bringing new antibiotics to market requires not only years of research and development but can also cost billions of dollars.[21] In response to this global crisis, the literature supports the use of GUMBOS (group of uniform materials based on organic salts) as antimicrobial agents. GUMBOS are a novel group of solid phase organic salts, typically using ionic liquid counter-ions; however, the melting point range of GUMBOS has been extended beyond that of ionic liquids, to the range of 25–250°C.[22] In contrast, the melting point for ionic liquids lies below 100°C. These compounds have uniquely tunable properties that can be incorporated into the salt via careful selection of counter-ions.[22] GUMBOS are stable, relatively non-toxic compared with their constituent compounds, and are as effective as or better than conventional antibiotic therapy against multi-antibiotic-resistant bacteria.[23,24] Antimicrobial GUMBOS are simple and inexpensive to synthesize from existing, well-known compounds such as antiseptics and antibiotics in their salt forms.

In this study, we strategically designed GUMBOS synthesized from octenidine and carbenicillin, a β-lactam antibiotic with high efficacy against various Gram-negative bacteria and with increased thermal and pH stability in solution.[25–27] However, carbenicillin is no longer administered due to toxicity issues at high concentrations.[28,29] By ion-exchanging the sodium ions on carbenicillin with octenidine, it is hypothesized that the toxicity situation will be improved.[23] The *in vitro* antibacterial efficacy of GUMBOS against *N. gonorrhoeae* ATCC 49226 and clinical isolates of *N. gonorrhoeae* was evaluated using disc diffusion susceptibility tests. Comparative analyses of octenidine/carbenicillin GUMBOS, their constituent parts, unreacted stoichiometric mixtures and current treatments for gonorrhoea confirmed the potential of this approach as an alternative therapy against the threat of antibiotic resistance. The toxicities of these GUMBOS were also evaluated.

Materials and methods

Disodium carbenicillin, methanol (MeOH) and DMSO were purchased from Sigma–Aldrich (Milwaukee, WI, USA). Octenidine dihydrochloride was purchased from TCI Chemicals (Japan). The cell viability MTT assay was purchased from Promega Corporation (Madison, WI, USA). Prepared agar plates (BD BBL Prepared Plated Media: GC II Agar with IsoVitalex™ Enrichment) and Oxoid™ antimicrobial susceptibility discs [ceftriaxone (30 μg), azithromycin (15 μg), doxycycline (30 μg) and blank discs; 6 mm] were purchased from Thermo Fisher Scientific (Pittsburgh, PA, USA). Parallel Artificial Membrane Permeability Assay (Gentest™ pre-coated PAMPA plate system) was purchased from Corning Incorporated (Tewksbury, MA, USA).

Synthesis and characterization of β-lactam-based GUMBOS

Synthesis and characterization of octenidine/carbenicillin ([OCT][CAR]) GUMBOS were performed using methods similar to those previously reported by Cole *et al.* (2015)[23] with slight modification. In the study reported here, octenidine/carbenicillin was easily synthesized using ion-exchange procedures that involved stirring stoichiometric amounts of octenidine dihydrochloride (OCT 2HCl) and disodium carbenicillin (Na_2 CAR) for 1 h at room temperature in deionized water (Figure 1). The resulting precipitate was washed several times with cold, deionized water and removed

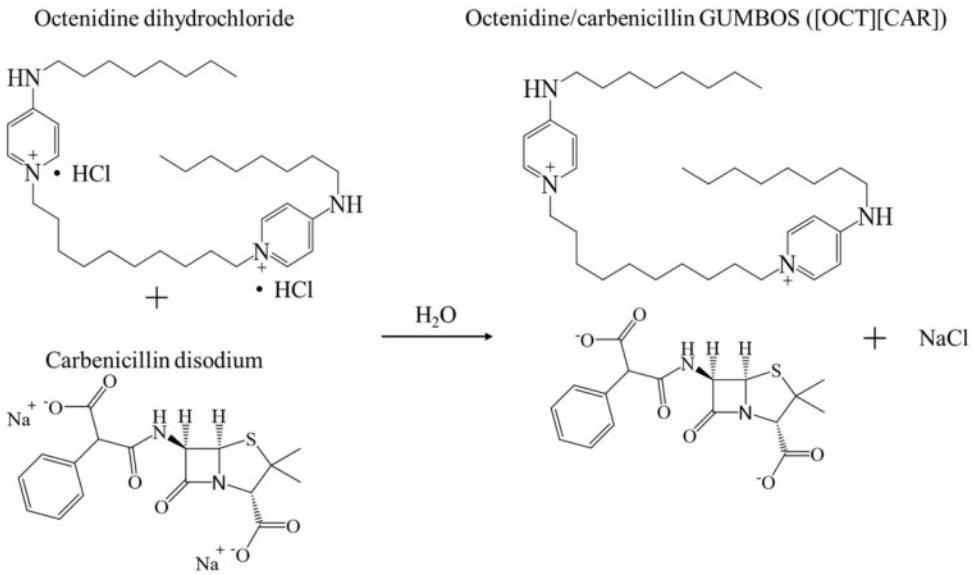

Figure 1. Synthesis and structures of precursor ions and octenidine/carbenicillin GUMBOS.

using lyophilization overnight. The structure of octenidine/carbenicillin was characterized using ^{1}H- and ^{13}C-NMR and Fourier transform infrared spectroscopy (FT-IR). Spectra are provided in Figures S1 to S3, respectively (available as Supplementary data at *JAC* Online). High-resolution mass spectrometry *m/z* is not reported as no useful spectrum was obtained, which can sometimes occur with carbenicillin.[30]

Predictive intestinal permeability

PAMPA was employed as an *in vitro* model of passive, transcellular permeation. In this technique, a 96-well microtitre plate is used as a donor plate and a membrane/acceptor compartment coated with structured tri-layers of phospholipids is placed on top; this configuration is referred to as 'sandwiched'. Known concentrations of octenidine/carbenicillin (100 μM in 1× PBS, 0.25% DMSO) were added to the donor plate while only buffer was placed in the acceptor plate. The assay was incubated for 5 h at room temperature and the acceptor plate was measured using a UV/Vis spectroscopy plate reader (Eppendorf PlateReader AF2200). Permeability coefficients (Pe) were calculated based on initial concentration in donor well (C_0), concentration in donor well at 5 h (C_D), concentration in acceptor well at 5 h (C_A), volumes of donor (V_D) and acceptor wells (V_A), well filter area (A, 0.3 cm^2) and incubation time (*t*, 18000 s), as calculated using the relationship in Equation 1.

$$\mathrm{Pe\ (cm/s)} = \frac{-\ln\left[\frac{C_A}{\frac{(C_D \times C_A)+(C_A \times V_A)}{V_D+V_A}}\right]}{A \times \left(\frac{1}{V_D}+\frac{1}{V_A}\right) \times t} \quad (1)$$

Antimicrobial susceptibility testing

Kirby–Bauer disc diffusion susceptibility tests were used to determine zones of inhibition (ZOIs) for *N. gonorrhoeae* (ATCC 49226) and three clinical isolates obtained from Louisiana State University Health Sciences Center New Orleans HIV Outpatient Clinic. Kirby–Bauer disc diffusion is one of the CDC's preferred methods of testing the susceptibility of *N. gonorrhoeae*.[31] Testing was performed according to CLSI recommended procedures.[31] In this susceptibility test, 6 mm diameter blank paper discs were impregnated with known quantities of antimicrobial drug followed by evaporation of solvent; however, ceftriaxone, azithromycin and doxycycline antimicrobial susceptibility discs were purchased from Thermo Fisher Scientific. Impregnated discs were placed onto prepared nutrient agar plates that were inoculated with *N. gonorrhoeae* to give a confluent lawn of growth. Suspensions of these various strains were prepared in accordance with a 1.0 McFarland standard. Inoculated agar plates were incubated for 20–24 h at 37°C in a 5% CO_2 atmosphere.

Cytotoxicity assay

In order to determine cell viability, a colorimetric MTT dye assay (Promega Corp., Madison, WI, USA) was used, employing the manufacturer's instructions, as an indicator of cytotoxicity of GUMBOS towards healthy (HeLa) cells. HeLa cells (ATCC CCL-2) grown in DMEM-reduced serum supplemented with 10% FBS were plated at a density of 1×10^5 cells/mL (10000 cells/well) in 96-well plates. Concentrations of therapeutic agents up to 500 μM (1% MeOH or DMSO) were doubly diluted in cell culture media and transferred to seeded cells. Cells were incubated for 15 min and 60 min at 37°C in a 5% CO_2 atmosphere. Cells treated with medium alone served as a negative control. At the end of the incubation period, 15 μL of MTT was added to each well and incubation continued for another hour. Absorbance was measured at 570 nm in a microplate spectrophotometer. All experiments were performed in quadruplicate. Cell viability as a percentage was determined as the ratio between treated cells and untreated (control) cells (taken as 100%). Reported values are the lethal concentrations able to kill 50% of the population of viable cells (IC_{50}).

Results and discussion

Characterization of octenidine/carbenicillin GUMBOS

Off-white solid, yield 90%. ^{1}H-NMR (400 Hz, DMSO-*d6*) δ 9.03 (br s, 1H), 8.64 (d, J = 8 Hz, 1H), 8.26 (dd, J = 8 Hz, 2H), 8.09 (dd, J = 8 Hz, 2H), 7.30–7.19 (m, 6H), 6.95 (dd, J = 8 Hz, 2H), 6.89 (dd, J = 8Hz, 2H), 5.29–5.25 (m, 2H), 4.08 (t, J = 8Hz, 4H), 3.81 (s, 1H), 3.53 (q, 2H), 3.24 (t, J = 4 Hz, 8 Hz, 4H), 1.71 (q, 4H), 1.55–1.51 (m, 7H), 1.43 (s, 3H), 1.32–1.22 (m, 31H), 0.85 (t, J = 8 Hz, 6H). ^{13}C-NMR (125 Hz, DMSO-*d6*) 170.45, 170.12, 169.23, 156.20, 142.89, 140.34, 135.58, 128.82, 128.72, 128.53, 127.62, 127.39, 125.71, 110.23, 104.33, 77.50, 70.77, 57.45, 56.05, 55.12, 42.43, 41.72, 30.72, 29.69, 28.72, 28.22, 28.19, 28.13, 28.11, 27.84, 27.40, 25.85, 24.82, 21.56, 13.41.

Predictive permeability

Octenidine 2HCl is a dicationic molecule with two chloride anions and this structure does not permeate through skin, mucous membranes, wounds or the placental barrier.[32] With an anion exchange metathesis from chloride ions to carbenicillin (a dianion), permeability increased significantly, which inevitably also increased bioavailability. Mean effective Pe for octenidine/carbenicillin was 3.78×10^{-6} (±0.85) cm/s, which falls in the range of high permeability. Pe values that are greater than 1.5×10^{-6} cm/s are defined as high permeability, whereas coefficients less than 1.5×10^{-6} cm/s are defined as low permeability.[33] This suggests that octenidine/carbenicillin and octenidine dihydrochloride behave differently chemically and thus GUMBOS may behave as a new ion pair when used therapeutically.

Antibacterial activity of octenidine/carbenicillin GUMBOS using disc diffusion

Antibacterial activity of octenidine/carbenicillin was compared with that of the individual parent compounds, unreacted stoichiometric mixtures and current therapeutic agents, ceftriaxone, azithromycin and doxycycline, using Kirby–Bauer disc diffusion (Table 1). Blank discs were loaded with 50 nmol of GUMBOS, respective constituent compounds and equivalent stoichiometric unreacted mixtures of octenidine and carbenicillin that are equimolar to the purchased ceftriaxone discs (Oxoid™, Thermo Fisher). After 20–24 h incubation, diameters of ZOIs were measured using a ruler. Ceftriaxone (30 μg, 50 nmol), azithromycin (15 μg, 20 nmol)

Table 1. Zones of inhibition for *N. gonorrhoeae* ATCC 49226 and clinical isolates

Test materials	Quantity of material (nmol)	Zone size, mm (±SD)	
		ATCC 49226	clinical isolates
Ceftriaxone	50	51±1	49±4
Azithromycin	20	40±1	36±4
Doxycycline	70	35±0.6	28±7
[OCT][CAR]	50	40±0.6	37±4
1:1 OCT:CAR	50	30±1	34±3
OCT 2HCl	50	9±1	9±0.3
Na_2 CAR	50	27±3	28±3

and doxycycline (30 μg, 70 nmol) had zone sizes within the susceptibility range set by CLSI for *N. gonorrhoeae* (ATCC 49226) and three clinical isolates tested. Antibacterial activity was improved for octenidine when chloride ions were exchanged for the antibiotic. Through synthesis of octenidine/carbenicillin GUMBOS, antibacterial activity exhibited an additive effect for *N. gonorrhoeae* (ATCC 49226) and clinical isolates. This effect was not seen, however, for an unreacted mixture of the two drugs for either *N. gonorrhoeae* (ATCC 49226) or clinical isolates. The ZOIs of octenidine/carbenicillin were also larger than or equal to azithromycin and doxycycline ZOIs for *N. gonorrhoeae* (ATCC 49226). When comparing ZOIs of octenidine/carbenicillin with those of azithromycin and doxycycline for the clinical isolate population, octenidine/carbenicillin exhibited equal efficacy to azithromycin while exhibiting superior activity to doxycycline, an antibiotic prescribed in the case of gonorrhoea and chlamydia coinfection.[34,35] As a result of these efficacy values, the antibacterial activity of octenidine/carbenicillin was shown to be bioequivalent to azithromycin. This is significant as resistance rates for azithromycin have increased worldwide. Among 57 countries reporting on azithromycin susceptibility, 28 (49%) reported >5% resistance.[36] In the USA, where clinical isolates were obtained, resistance increased significantly from 2014 to 2019; the percentage of isolates with elevated resistance rates to azithromycin increased from 2.5% to 4.6%.[37] Ceftriaxone produced large ZOIs as the clinical population still remains susceptible to it in the USA (only 0.2% of isolates had elevated resistance to ceftriaxone in 2017).[37]

Cytotoxicity of octenidine and carbenicillin in combination and as GUMBOS

Following characterization and antimicrobial efficacy testing, GUMBOS were employed *in vitro* to assess relative cytotoxicity as compared with the stoichiometric, unreacted mixture and octenidine dihydrochloride. When tested *in vitro*, β-lactam antibiotics are known to be highly non-toxic.[38] Octenidine dihydrochloride has also shown low cytotoxic potential when tested against human primary gingival fibroblasts and human primary nasal epithelial cells.[39] Octenidine has also been shown to be an effective mouth rinse for substantially reducing oral bacterial counts.[18,40] For a general approximation of systemic toxicity and possible application beyond mouthwash, such as a vaginal douche, cervical cells were used. Cytotoxicity was assayed at 15 and 60 min because oral rinses are most beneficial if an individual does not drink liquids at least 15 to 60 min after use. If also used to eradicate infections of the cervix, this time frame could allow ample opportunity for the drug to remain in contact with cervical tissue before being removed from the body. The IC_{50} values for OCT 2HCl, unreacted mixture and octenidine/carbenicillin are reported in Table 2.

After 15 min of incubation, octenidine/carbenicillin showed lower toxicity than the stoichiometric, unreacted mixture; however, OCT 2HCl was the most toxic to cervical cells. After 60 min of incubation, the toxicity of the unreacted mixture of OCT 2HCl and Na_2 CAR was still lower than that of GUMBOS. While cytotoxicity potential varied between GUMBOS and the parent compound mixture, the unreacted mixture of the two antimicrobial compounds still contains the two sodium ions, which may aggravate hypertension or congestive heart failure.[41,42] GUMBOS would, therefore, be inherently safer as the sodium ions are removed entirely and replaced with octenidine.

Table 2. Acute cytotoxicity (IC_{50}) of octenidine/carbenicillin GUMBOS for 15 and 60 min against HeLa cells

	IC_{50} (mg/L)	
Test materials	15 min	60 min
OCT 2HCl	37.4±2.8	16.1±0.7
1:1 OCT:CAR	52.8±4.8	26.6±0.9
[OCT][CAR]	44.1±2.5	23.1±0.2

Octenidine/carbenicillin GUMBOS to reduce transmission of gonorrhoea

In 2019, a new paradigm for the transmission of extragenital *N. gonorrhoeae* emerged. Oropharyngeal infections were noted in the absence of urogenital infections[43] and it was hypothesized that kissing or saliva exchange could be an unrecognized means of transmission.[4,44] It was proposed that antiseptic mouthwashes might offer a condom-free control strategy.[5] In this regard, Chow *et al.*[16] reported that use of Listerine® mouthwash as a gargle could significantly reduce the amount of *N. gonorrhoeae* in the oropharynx of MSM. As a result of this study, we propose that octenidine/carbenicillin GUMBOS could be incorporated into a mouthwash for this purpose.

The constituent components of our GUMBOS, octenidine and carbenicillin, have established safety profiles. Octenidine is currently formulated as a mouthwash and carbenicillin, like most β-lactam antibiotics, is relatively non-toxic to mammalian cells, as shown in Table 2. We also believe that our GUMBOS, formulated as pessaries or suppositories, could be used to reduce colonization in other anatomical sites such as the vagina and rectum.

Conclusions

As the threat of antibiotic resistance in *N. gonorrhoeae* increases and oropharyngeal cases become more difficult to treat, alternative therapies are greatly needed. This study suggests that octenidine/carbenicillin GUMBOS may be a viable alternative therapy for prevention and minimization of *N. gonorrhoeae* transmission. GUMBOS were easily synthesized using ion-exchange reactions in deionized water. Octenidine/carbenicillin was found to be bioequivalent to azithromycin and doxycycline, as determined by Kirby–Bauer disc diffusion assays. Moreover, octenidine/carbenicillin exhibited higher efficacy than the constituent parent compounds and unreacted mixtures. Cytotoxicity results showed that octenidine/carbenicillin was also non-toxic towards cervical cells. This approach of fashioning antimicrobial agents into GUMBOS may offer an alternative approach to current drug therapies for gonorrhoea and have further implications for topical prevention strategies.

Acknowledgements

We thank Karen McDonough for her assistance with cytotoxicity studies and Dr Stephanie Taylor for providing the clinical isolates of *N. gonorrhoeae*.

Funding

This research is based upon work supported in part by the Louisiana Biomedical Collaborative Research Program, a National Science Foundation Bridge to the Doctorate Fellowship (grant # NSF HRD-1301957) to Kelsey M. Lopez, and Philip W. West Endowment and National Science Foundation (grant # CHE-1905105).

Transparency declarations

None to declare.

Disclaimer

Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Supplementary data

Figures S1 to S3 are available as Supplementary data at *JAC* Online.

References

1 WHO. Human reproduction programme. Scientists Warn That Antibiotic-Resistant Gonorrhoea is on the Rise. 2017.

2 CDC. Sexually Transmitted Disease Surveillance 2018. 2019. https://www.cdc.gov/std/stats18/default.htm

3 Whittles LK, Didelot X, Grad YH *et al*. Testing for gonorrhoea should routinely include the pharynx. *Lancet Infect Dis* 2018; **18**: 716–7.

4 Chow EPF, Cornelisse VJ, Williamson DA *et al*. Kissing may be an important and neglected risk factor for oropharyngeal gonorrhoeae: a cross-sectional study in men who have sex with men. *Sex Transm Infect* 2019; **95**: 516–21.

5 Hook EW, Bernstein K. Kissing, saliva exchange, and transmission of *Neisseria gonorrhoeae*. *Lancet Infect Dis* 2019; **19**: e367–9.

6 Weinstock H, Workowski KA. Pharyngeal gonorrhea: an important reservoir of infection? *Clin Infect Dis* 2009; **49**: 1798–800.

7 Bro-Jorgensen A, Jensen T. Gonococcal pharyngeal infections: report of 110 cases. *Br J Vener Dis* 1973; **49**: 491–9.

8 Pattani A. A dangerous, "silent reservoir" for gonorrhea: the throat. *The New York Times* 2017: D5.

9 Lewis DA. Will targeting oropharyngeal gonorrhoea delay the further emergence of drug-resistant *Neisseria gonorrhoeae* strains? *Sex Transm Infect* 2015; **91**: 234–7.

10 Miari VF, Ison CA. Is there a role for topical antiseptics in the treatment of gonorrhoea? *Sex Transm Infect* 2017; **93**: 79–80.

11 CDC. Sexually transmitted diseases treatment guidelines, 2015. *MMWR Recomm Rep* 2015; **64**: 1–37.

12 Unemo M, Golparian D, Hestner A. Ceftriaxone treatment failure of pharyngeal gonorrhoea verified by international recommendations, Sweden, July 2010. *Eurosurveill* 2011; **16**: 19792.

13 PHE. UK case of *Neisseria gonorrhoeae* with high-level resistance to azithromycin and resistance to ceftriaxone acquired abroad. Health Protection Report Advanced Access Report, 2018.

14 Shah PM, Isaacs RD. Ertapenem, the first of a new group of carbapenems. *J Antimicrob Chemother* 2003; **52**: 538–42.

15 Herrold RD, Culver H. The treatment of acute gonorrhea with antiseptics in gelatin. *JAMA* 1927; **88**: 459–60.

16 Chow EPF, Howden BP, Walker S *et al*. Antiseptic mouthwash against pharyngeal *Neisseria gonorrhoeae*: a randomised controlled trial and an in vitro study. *Sex Transm Infect* 2017; **93**: 88–93.

17 Baskaran SA, Upadhyay A, Upadhyaya I *et al*. Efficacy of octenidine hydrochloride for reducing *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* on cattle hide. *Appl Environ Microbiol* 2012; **78**: 4538–41.

18 Patters MR, Anerud K, Trummel CL *et al*. Inhibition of plaque formation in humans by octenidine mouthrinse. *J Periodontal Res* 1983; **18**: 212–9.

19 Stahl J, Braun M, Siebert J *et al*. The percutaneous permeation of a combination of 0.1% octenidine dihydrochloride and 2% 2-phenoxyethanol (octenisept®) through skin of different species in vitro. *BMC Vet Res* 2011; **7**.

20 Hubner N-O, Siebert J, Kramer A. Octenidine dihydrochloride, a modern antiseptic for skin, mucous membranes and wounds. *Skin Pharmacol Physiol* 2010; **23**: 244–58.

21 Tufts Center for the Study of Drug Development. Cost to develop and win marketing approval for a new drug is $2.6 billion. 2014.

22 Warner IM, El-Zahab B, Siraj N. Perspectives on moving ionic liquid chemistry into the solid phase. *Anal Chem* 2014; **86**: 7184–91.

23 Cole MR, Hobden JA, Warner IM. Recycling antibiotics into GUMBOS: a new combination strategy to combat multi-drug-resistant bacteria. *Molecules* 2015; **20**: 6466–87.

24 Cole MR, Li M, Jadeja R *et al*. Minimizing human infection from *Escherichia coli* O157:H7 using GUMBOS. *J Antimicrob Chemother* 2013; **68**: 1312–8.

25 Bodey GP, Whitecar JP, Middleman E *et al*. Carbenicillin therapy for *Pseudomonas* infections. *JAMA* 1971; **218**: 62–6.

26 GoldBio. Carbenicillin (Disodium). https://www.goldbio.com/product/960/carbenicillin-disodium.

27 Grisp. Carbenicillin (disodium salt). https://grisp.pt/produto/carbenicillin-disodium-salt/.

28 McClure PD, Casserly JG, Monsier C *et al*. Carbenicillin-induced bleeding disorder. *Lancet* 1970; 1307–8.

29 Waisbren BA, Evani SV, Ziebert AP. Carbenicillin and bleeding. *JAMA* 1971; **217**: 1243.

30 Mills T 3rd, Robertson JC, Matchett CC *et al*. *Instrumental Data for Drug Analysis*. Elsevier, 1992.

31 CDC. Antimicrobial Resistance Susceptibility Testing: Disk Diffusion Susceptibility Testing. 2018. www.cdc.gov/std/gonorrhea/lab/diskdiff.htm.

32 Weissenbacher ER, Klemm A, Baumgartner L *et al*. Octenisept®: study of transplacental passage in an in vitro perfusion model with human placenta (in German). *Int J Exp Clin Res* 1997; **10**: 1–6.

33 Corning. Corning Gentest PAMPA Plate System: Frequently Asked Questions, 2012. 2013. https://www.corning.com/catalog/cls/documents/sell-sheets/CLS-DL-GT-023_REV1_DL.pdf

34 Dalke B, Ivers T, O'Brien KK *et al*. Gonorrhea: treatment and management considerations for the male patient. *US Pharmacist* 2016; **41**: 41–4.

35 Mayor MT, Roett MA, Uduhiri KA. Diagnosis and management of gonococcal infections. *Am Fam Physician* 2012; **86**: 931–8.

36 WHO. Gonococcal antimicrobial susceptibility. Report on Global Sexually Transmitted Infection Surveillance. 2018.

37 CDC. Sexually Transmitted Disease Surveillance 2017. 2018. https://www.cdc.gov/std/stats17/default.htm

38 Goo K-S, Sim T-S. Designing new β-lactams: implications from their targets, resistance factors and synthesizing enzymes. *Curr Comput Aided Drug Des* 2011; **7**: 53–80.

39 Schmidt J, Zyba V, Jung K *et al*. Cytotoxic effects of octenidine mouth rinse on human fibroblasts and epithelial cells – an *in vitro* study. *Drug Chem Toxicol* 2016; **39**: 322–30.

40 Dogan AA, Cetin ES, Hussein E *et al*. Microbiological evaluation of octenidine dihydrochloride mouth rinse after 5 days' use in orthodontic patients. *Angle Orthod* 2009; **79**: 766–72.

41 Kester M, Karpa KD, Vrana KE. Treatment of infectious diseases. *Elsevier's Integr Rev Pharmacol* 2012; 41–78.

42 Hosenpud JD, Greenberg BH. *Congestive Heart Failure: Pathophysiology, Diagnosis, and Comprehensive Approach to Management*. Springer, 2013.

43 Cornelisse VJ, Bradshaw CS, Chow EPF *et al*. Oropharyngeal gonorrhea in absence of urogenital gonorrhea in sexual network of male and female participants, Australia, 2018. *Emerg Infect Dis* 2019; **25**: 1373–6.

44 Fairley CK, Zhang L, Chow EPF. New thinking on gonorrhoea control in MSM: are antiseptic mouthwashes the answer? *Curr Opin Infect Dis* 2018; **31**: 45–9.