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Evaluation of the potential of colicins to prevent extraluminal contamination of urinary catheters by *Escherichia coli*

Sandra M. Roy*, Margaret A. Riley

University of Massachusetts Amherst, 611 N. Pleasant Street Morrill Science Center III Amherst, MA 01003

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ABSTRACT

The feasibility of using colicins to create an antimicrobial lubricant to prevent extraluminal catheter contamination during urinary catheter insertion was assessed. Levels of resistance of uropathogenic *Escherichia coli* to antibiotics and colicins were compared. The results showed that antibiotics and colicins possess similar frequencies of resistance to a single drug, whereas colicins exhibit significantly lower levels of multidrug resistance (22%) than antibiotics (42%). Colicins and antibiotics showed complementary inhibitory activity, with each targeting different subsets of pathogenic isolates. The collateral impact of these two antimicrobials on genera that are members of the fecal/vaginal/urinary microbiome was assessed, with colicins showing significantly less collateral damage than antibiotics. Using a novel colicin, SR4, minimum inhibitory concentrations (MICs) for a panel of 30 uropathogenic isolates were determined and showed that SR4 achieved the same antimicrobial efficacy as gentamicin using 20-30% less drug. An SR4-impregnated catheter lubricant was created and its ability to prevent extraluminal urinary catheter contamination *in vitro* was demonstrated. These data indicate that a colicin-impregnated lubricant may provide a viable prophylactic option for preventing catheter-associated urinary tract infections.

1. Introduction

Catheter-associated urinary tract infections (CAUTI) are the leading cause of nosocomial infections in the US [1,2], with nearly half a million events reported annually [3]. Insertion of a urinary catheter often leads to infection by breaking down the body's natural defense mechanisms and allowing external bacteria access to the bladder. Extraluminal contamination is most frequent, as bacteria are introduced into the bladder during catheter insertion or migrate into the body via the catheter [4]. Once contaminated, the catheter itself provides an ideal surface on which bacteria can adhere and form biofilms. Colonization of the catheter occurs almost immediately following catheterization [2].

Many isolates of uropathogenic *Escherichia coli* (UPEC), the major cause of nosocomial CAUTI, are resistant to more than one of the antibiotics generally used in their treatment [1]. Considering the high levels of resistance to antibiotics and rapidly increasing rates of catheterization required in an aging US population, numerous studies have explored methods to prevent contamination during the catheterization process and subsequent catheter-based biofilm formation [5]. Most of these therapeutic or intervention methods have proven ineffective, primarily due to the ease and speed with which uropathogens form biofilms on and in the catheter [4,5]. Although the use of silver-coated catheters was an initially promising approach, it was determined to be ineffective based upon a meta-analysis of 22 clinical studies [5]. The use of antibiotic-coated catheters to prevent or delay CAUTI onset in hospitalized patients has been explored. However, as CAUTI biofilms are intrinsically resistant to most antibiotics and their use contributes to increasing rates of resistance, this approach is less promising [5].

Given the lack of treatment options, there is significant interest in developing effective prophylactic measures to prevent CAUTI. Our approach utilizes a family of bacterial toxins, the bacteriocins. These are broadly defined as proteins and peptides produced by bacteria to inhibit the growth of closely related strains [6]. The present study focuses on the bacteriocins produced by *E. coli*, the colicins, which possess a narrow spectrum of activity, usually only active against other isolates of *E. coli*. Colicins possess characteristics that make them promising candidates in CAUTI prophylaxis, including high levels of activity against *E. coli*, the ability to adhere

* Corresponding author: Sandra M. Roy, PhD, Present address: College Street Consulting, 100 College Street South Hadley, MA 01075.
E-mail addresses: CollegeStConsulting@gmail.com, Sandra.Marie.Roy@gmail.com (S.M. Roy), Riley@bio.umass.edu (M.A. Riley).

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0924-8579/

Table 1
Characterized colicins utilized in this study.

Colicin Name	Mode of Action	Receptor
Colicin E1	Pore-former	BtuB
Colicin E4	16s Rnase	BtuB
Colicin E7	Dnase	BtuB
Colicin N	Pore-former	OmpF

to a catheter and retain activity, the ability to inhibit quiescent bacteria in biofilms, and their ease of genetic manipulation, gene expression, and protein purification.

This study was conducted to assess the potential of colicins as novel antimicrobial agents to prevent extraluminal contamination of urinary catheters by *E. coli*. The efficacy of colicins against *E. coli* isolates from patients with CAUTI and/or UTIs, and their corresponding lack of activity against beneficial bacteria normally resident in a healthy microbiome are described. A novel colicin-based catheter lubricant formulation is created and applied to Foley catheters to assess whether it inhibits extraluminal catheter contamination by *E. coli* under a variety of medically relevant conditions of use. These data indicate that colicin-based lubricating jelly reduces or eliminates *E. coli*-based extraluminal contamination of catheters. Finally, considering other bacterial species implicated in CAUTI also produce bacteriocins, it should be possible to create a cocktail of bacteriocins that provide powerful anti-CAUTI prophylaxis against all clinically relevant CAUTI bacterial pathogens.

2. Materials & methods

2.1. Bacterial isolates

Cooley Dickinson Hospital (Northampton, MA) provided bacterial isolates from human urine (clean-catch, collection system, or in-dwelling catheter) during 2011. These isolates span the following genera: *Escherichia* (n=100) (specifically *E. coli*), *Pseudomonas* (n=40), *Klebsiella* (n=40), *Acinetobacter* (n=19), *Proteus* (n=41), *Enterococcus* (n=20), *Staphylococcus* (n=40), *Citrobacter* (n=23), and *Enterobacter* (n=33). All patient data were expunged from the samples.

Known colicin-producing strains were provided by Anthony Pugsley [7,8] and are noted in Table 1. Novel colicin-producing strains (Figure 1B) were provided from the following collections:

UTI23, UTI45, RS107: Richard Goldstein, Boston University [9] (human urine)
CD-114: Cooley Dickinson Hospital (Northampton, MA) (human urine)
TA125, TA176, TA243: David Gordon, Australia National University (fecal samples from live trapped mammals).

A colicin-sensitive control strain, BZB1011, was used to detect and standardize colicin activity. Clinical Laboratory Sciences Institute (CLSI) standard *E. coli* strain ATCC25922 was used as a control in all minimum inhibitory concentration (MIC) experiments.

Sequence determination of a plasmid isolated from strain TA243 [10] revealed a novel 576 AA colicin protein, which was named colicin SR4.

2.2. Resistance screening and collateral damage assessment

2.2.1. Antibiotic resistance testing

Antibiotic resistance profiles for the 100 *E. coli* isolates were provided by Cooley Dickinson Hospital (Northampton, MA) and were performed using a VITEK II analyzer (bioMerieux) under standard clinical laboratory protocols. Antibiotics tested are shown in Table 2.

Table 2
Antibiotics Empirically Prescribed for the Treatment of Urinary Tract Infections.

Antibiotic (abbreviation)	**Class**
Gentamicin (gm)	Aminoglycoside
Ampicillin (am)	Penicillin
Cefazolin (cz) Cefoxitin (fox) Ceftriaxone (cro) Cefepime (fep)	Cephalosporin
Levofloxacin (lev) Ciprofloxacin (cip)	Fluoroquinolone
Nitrofurantoin (ft)	Nitrofurans
Ampicillin/Sulbactam (sam)	Penicllin/beta-lactam inhibitor
Trimethoprim/ Sulfamethoxazole (sxt)	Sulfonamide

2.2.2. Colicin lysate production

Crude colicin lysates were prepared from known colicin-producing strains using standard procedures [11]. The resulting lysates were collected and stored at 4°C until use. Lysates were tested for colicin activity via production of a zone of inhibition on a colicin-sensitive *E. coli* lawn (BZB1011).

2.2.3. Colicin resistance testing

Resistance to colicins was determined using a standard bacteriocin patch assay [10]. Colicin lysates were spotted on lawns of bacterial isolates of interest then incubated overnight. Lawns were recorded as sensitive if the colicin produced a zone of inhibition and resistant if no zone was observed. Recent studies have correlated zones of inhibition with MICs [12].

2.3. Colicin partial purification

2.3.1. Partial purification

Crude colicin lysates were filtered through a 30 kDa Centricon Plus-70 (EMD Millipore) molecular weight filter in a swinging bucket centrifuge following the manufacturer's protocol [13]**.** A 30 kDa filter was chosen because the filter should be 2-3 times smaller than the protein of interest [13]. The goal of this step was to remove impurities and concentrate the colicin in the sample.

2.3.2. Protein quantification

Partially purified colicin protein concentrations were determined using SDS-PAGE gel image analysis (ImageJ) and direct comparison with known concentrations of bovine serum albumin (BSA) [14].

2.4. Minimum inhibitory concentrations (MICs)

MICs were determined according to the CLSI guidelines using the direct colony suspension method [15,16].

2.5. Creation and testing of colicin-impregnated lubricant

2.5.1. Colicin-impregnated lubricant

Colicin-impregnated catheter lubricant was created by adding partially purified colicin SR4 to Covidien sterile lubricant (in 5 g individual foil packs) for a final colicin concentration of 2.85 μg/mL. This lubricant is water-soluble, routinely used in catheterization, comes in pre-weighed individual packets, and has proven the easiest to work compared with four other lubricants tested (E-Z lubricating jelly [Medline], Surgilube surgical lubricant [Savage Laboratories], Lubricating jelly [McKesson], and Lubricating jelly [HR]).

2.5.2. Prevention of contamination

1 cm sections of Bardia™ All-silicone Foley catheters were dipped in Covidien lubricant or colicin-impregnated lubricant. Catheter sections were then placed into Luria Broth containing 10^3 cfu/mL of *E. coli* and incubated for either 10 min or 24 h. Cell densities were determined in the *E. coli* solutions by cell count following incubation with catheter sections. Catheter sections were then rolled onto an LB Agar plate to detect the presence of *E. coli*. Each experiment was conducted in triplicate.

2.5.3. Stability

Aliquots of partially purified colicin SR4 and SR4-impregnated lubricant were stored at each of the following temperatures to mimic potential storage and use conditions: −80°C, −20°C, 4°C, 25°C, 37°C, and 40°C. Exposure to 40°C simulates accelerated aging, a standard measure of shelf-life for medical devices [17]. The initial pH of partially purified colicin SR4 and SR4-impregnated lubricant was determined to be 7. pH 4 and 9 versions of the protein and lubricant were made with the addition of 0.1 N sodium hydroxide (NaOH) or 0.1 N hydrochloric acid (HCl). All three pH versions were incubated for 1 h at 37°C and activity determined by plating onto a colicin-sensitive lawn of *E. coli* (BZB1011).

2.6. Statistical analysis

Statistical analysis was performed using GraphPad Software.

P-values were calculated using an unpaired two-tailed Student's t-test, and chi-square analysis. *P*-values of ≤ 0.05 were considered statistically significant.

3. Results and Discussion

3.1. Sensitivity of E. coli *isolates to antibiotics and colicins*

The goal of this study was to compare the inhibitory effect of colicins on the growth of *E. coli* isolated from human urine with that of conventional antibiotics. Antibiotic resistance profiles for 100 such isolates were provided by Cooley Dickinson Hospital (Figure 1A). Although clinically relevant resistance thresholds are determined for each antibiotic, it is generally accepted that once resistance levels reach 20-30%, such a drug should no longer be considered a first-line therapeutic [18]. Colicin resistance profiles were determined for these same 100 *E. coli* isolates (Figure 1B). An unpaired t-test revealed no significant difference between the resistance levels for colicins and antibiotics among this sample of isolates (P=1.00) (Figure 1).

The levels of antibiotic resistance observed among these isolates are not surprising. Numerous prior studies have revealed that uropathogenic *E. coli* have evolved or acquired resistance to most of the antibiotics commonly used in their treatment [1]. The levels of colicin resistance observed among these isolates is similar to those detected in numerous prior screens for colicin resistance [19]. Recent studies have compared levels of antibiotic resistance to phages and pyocins [1,20] but, to our knowledge, this study is the first direct comparison of levels of antibiotic and colicin resistance.

3.1.1. Antibiotic and colicin multidrug resistance

The prevalence of multidrug resistance (MDR), particularly for uropathogens, is a topic of great concern. On average, 70% of catheterized hospital patients are estimated to receive antibiotic treatment during their stay for conditions unrelated to UTI/CAUTI

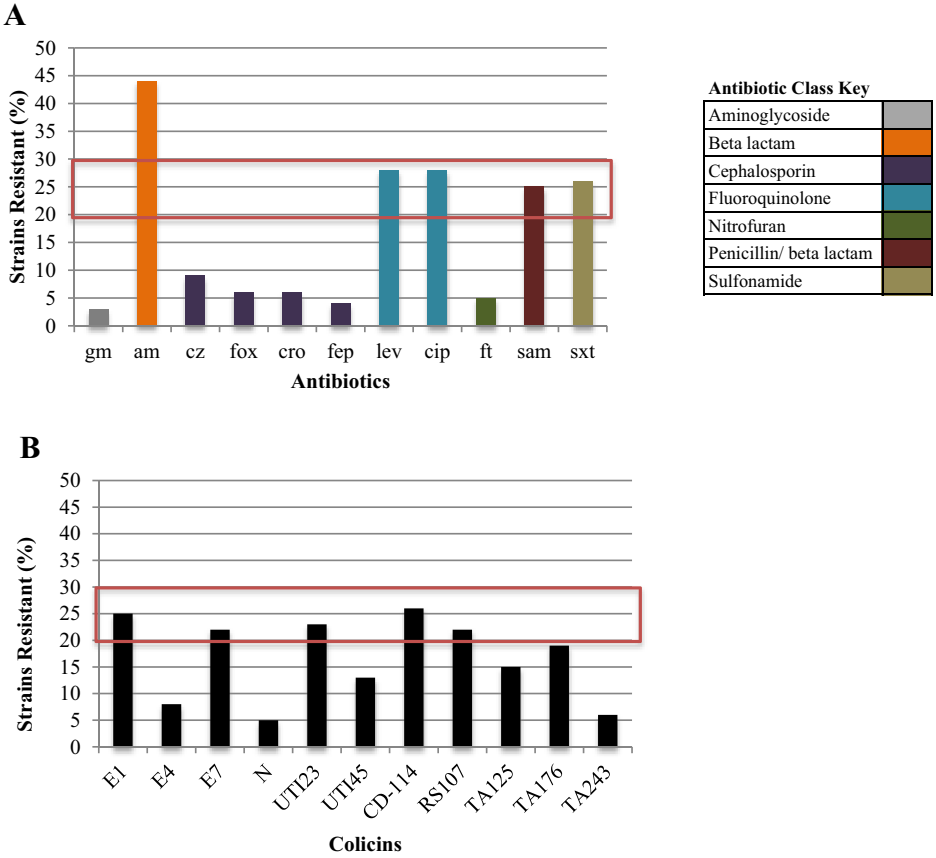

Fig. 1. Resistance of *E. coli*. (A) resistance to antibiotics empirically prescribed for the treatment of urinary tract infections and (B) resistance to colicins effective against *E. coli*. The resistance threshold (20-30%) is denoted by a red box.

Table 3
Activity of antibiotics of interest against 9 genera of bacteria.

Lawn Species (n=)	Antibiotics										
	Ampicillin	Ciprofloxacin	Ceftriaxone	Cefazolin	Cefepime	Cefoxitin	Nitrofurantoin	Gentamicin	Levofloxacin	Ampicillin/Sulbactam	Trimethoprim/sulfamethoxazole
Total Pseudomonas killed (40)	1	25	1	1	34	1	0	36	25	1	1
% of Pseudomonas killed	3%	63%	3%	3%	85%	3%	0%	90%	63%	3%	3%
Total Klebsiella killed (40)	1	39	37	34	37	38	20	40	39	35	39
% of Klebsiella killed	3%	98%	93%	85%	93%	95%	50%	100%	98%	88%	98%
Total Acinetobacter killed (12)	2	10	4	1	10	3	1	12	10	11	11
% of Acinetobacter killed	17%	83%	33%	8%	83%	25%	17%	100%	83%	92%	92%
Total Proteus killed (41)	24	20	40	35	40	37	0	25	20	30	24
% of Proteus killed	59%	49%	98%	85%	98%	90%	0%	61%	49%	73%	59%
Total Bacillus killed (20)	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
% of Bacillus killed											
Total Enterococcus killed (40)	33	NT	NT	NT	NT	NT	34	NT	21	33	NT
% of Enterococcus killed	83%						87%		66%	100%	
Total Staphylococcus killed (40)	NT	NT	21	NT	NT	NT	37	40	20	21	36
% of Staphylococcus killed			53%				97%	100%	50%	53%	90%
Total Citrobacter killed (23)	NT	23	23	10	23	10	18	23	23	NT	23
% of Citrobacter killed		100%	100%	43%	100%	43%	90%	100%	100%		100%
Total Enterobacter killed (33)	NT	29	33	0	33	0	3	29	29	NT	25
% of Enterobacter killed		88%	100%	0%	100%	0%	10%	88%	88%		76%
Total Strains Killed	61	146	159	81	177	89	113	205	187	131	159
Total Strains Tested	180	196	236	196	196	196	262	236	268	213	236
Total % of Strains Killed	**34%**	**74%**	**67%**	**41%**	**90%**	**45%**	**43%**	**87%**	**70%**	**62%**	**67%**

[1]. This antibiotic treatment selects for antibiotic resistance in the majority of species comprising the patients' microbiome, thereby increasing levels of resistant organisms in the patient and increasing the probability that an antibiotic-resistant pathogen will be the cause of contamination during catheter insertion and subsequent CAUTI. Therefore, the prevalence of MDR to antibiotics and colicins was compared for the isolates in our study.

MDR is defined as resistance to more than one class of antibiotics or colicins (based on colicin mode of action). **Supplemental Figure 1** provides MDR data for the same 100 *E. coli* isolates discussed above, both for antibiotics and colicins with known modes of action. These data reveal a significant difference in the levels of antibiotic (42%) vs. colicin (22%) MDR. To our knowledge, no previous studies have directly compared MDR levels for these unrelated classes of antimicrobials.

3.1.2. Antibiotic and colicin combinations

The killing ability of colicins and antibiotics was found to be complementary, with the two types of antimicrobials targeting different pathogen subsets (**Supplemental Figure 1**). This is likely due to the high cost of maintaining colicin resistance in strains that also bear the cost of maintaining antibiotic resistance. Given the intriguing finding that isolates resistant to antibiotics are often more sensitive to colicins, the impact of combining antibiotics and colicins to reach higher levels of efficacy was assessed against this sample of isolates. The percentage of 100 *E. coli* isolates inhibited when exposed to pairs of antibiotics and colicins is shown in **Supplemental Table 1**. Four of the antibiotic combinations and one colicin combination inhibited 100% of the isolates examined. However, when antibiotics were combined with colicins, 52 pairs of drugs inhibited 100% of the isolates. This result echoes the benefits of combining drugs seen in previous studies [19,20].

3.2. Colicins are target-specific; reducing the incidence of collateral damage to the microbiome

The key role of the gut microbiome in supporting human health is clear, as is the extensive collateral damage caused to the microbiome by the use of broad spectrum antibiotics. Even the urinary tract, which was once thought to be sterile, possesses a urinary microbiome, although its function is not yet well understood [21,22]. Given our ignorance on which bacterial species comprise these, and other, critical microbiomes in and on the human body and the roles they serve in supporting health, it is important that collateral damage becomes a key design criterion in developing novel antimicrobials. This is where traditional antibiotics fall short.

Bacteriocins do not exhibit the breadth of activity of antibiotics. Colicins' activity is generally restricted to *E. coli* or its closest relatives. In contrast, many conventional antibiotics act against a diversity of Gram-positive and/or Gram-negative bacterial taxa. The differential impact on the microbiome of the highly selective targeting of bacteriocins compared with broadly acting antibiotics is assumed to be significant [2]. To explore this, 11 antibiotics and 11 colicins were assayed for activity against a panel of 296 clinical isolates from 9 bacterial genera representative of members of the gut microbiome, fecal flora, and/or implicated as causative agents of UTIs [23]. The presence of several of these genera in the urinary microbiome has been recently described [21].

As expected, antibiotic sensitivity was significant, ranging from 34 to 90% (Table 3), whereas colicin sensitivity was far more limited, ranging from 0 to 11% (Table 4). Most of the colicin sensitivity detected was due to a single colicin (UTI45) inhibiting isolates from two genera (*Klebsiella* and *Citrobacter*), both of which are relatively closely related to *E. coli*. When this colicin (UTI45) was excluded from the analysis, the range of colicin sensitivities dropped to 0 to 4%. The colicins inhibited an average of 59% fewer isolates

Table 4
Activity of colicins of interest against 9 genera of bacteria.

Lawn Species (n=)	Colicins										
	E1	E4	E7	N	UTI23	UTI45	CD-114	RS107	TA125	TA176	TA243
Total Pseudomonas killed (40)	0	0	0	0	0	0	0	0	0	0	0
% of Pseudomonas killed	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Total Klebsiella killed (40)	0	0	0	0	0	16	0	0	0	0	0
% of Klebsiella killed	0%	0%	0%	0%	0%	40%	0%	0%	0%	0%	0%
Total Acinetobacter killed (19)	1	1	0	0	0	1	0	0	1	1	1
% of Acinetobacter killed	5%	5%	0%	0%	0%	5%	0%	0%	5%	5%	5%
Total Proteus killed (41)	0	0	0	0	0	0	0	0	0	0	0
% of Proteus killed	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Total Bacillus killed (20)	0	0	0	0	0	0	0	0	0	0	0
% of Bacillus killed	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Total Enterococcus killed (40)	0	0	0	0	0	0	0	0	0	0	0
% of Enterococcus killed	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Total Staphylococcus killed (40)	0	0	0	0	0	0	0	0	0	0	0
% of Staphylococcus killed	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
Total Citrobacter killed (23)	0	5	1	4	0	12	0	0	1	4	5
% of Citrobacter killed	0%	22%	4%	17%	0%	52%	0%	0%	4%	17%	22%
Total Enterobacter killed (33)	4	5	0	0	0	3	0	0	1	0	3
% of Enterobacter killed	12%	15%	0%	0%	0%	9%	0%	0%	3%	0%	9%
Total Strains Killed (259)	**5**	**11**	**1**	**4**	**0**	**32**	**0**	**0**	**3**	**5**	**9**
Total % of Strains Killed	**2%**	**4%**	**0.3%**	**1%**	**0%**	**11%**	**0%**	**0%**	**1%**	**2%**	**3%**

Table 5
Minimum Inhibitory Concentrations.

MIC Target	Units	Metric	Antimicrobial		
			Colicin SR4	Colicin E4	Gentamicin
N/A	N/A	Molecular Weight (kDa)	61.4	57.8	0.48
CLSI Standard *E. coli* strain ATCC25922	ug/mL	MIC	8.0	20.0	0.5
30 *E. coli* isolated from urine	ug/mL	Average MIC	6.6	10.4	1.5
		MIC Range	<.03 to >16	<.04 to >20	0.5 to >32
		MIC50	4.0	5.0	1.0
		MIC90	>16	>20	2.0
30 *E. coli* isolated from urine (same as above)	nM	Average MIC	107.5	180.0	3,140.7
		MIC Range	<2 to >260.6	<1 to >346	1,046.9 to >8,375
		MIC50	65.2	86.5	2,093.8
		MIC90	260.6	>346	4,187.5

in this sample than the antibiotics (x^2=214.76, P<0.0001). To our knowledge, there are no comparable estimates of reduced collateral killing of the microbiome with colicins compared with antibiotics.

4. Creation and testing of a colicin-impregnated lubricant

4.1. Activity of partially purified colicins SR4 and E4 against E. coli *isolated from urine*

Colicin sensitivity screening identified a colicin-producing strain (TA243) that killed 94% of the *E. coli* isolates assayed. A large-construct plasmid isolation of strain TA243 revealed a plasmid that encoded a novel colicin, SR4, which was used in studies designed to assess the potential for colicins to prevent catheter contamination.

MICs were determined for partially purified colicin SR4, colicin E4, and gentamicin against a panel of 30 *E. coli* isolated from urine (a subset of the 100 isolates tested above) (Table 5). MICs of the target isolates were lower for gentamicin than for colicins.

Reporting colicin MICs in units of μg/mL does not adequately capture their antimicrobial capabilities. Most antibiotics are smaller than 0.9 kDa, with gentamicin weighing 0.479 kDa [24]. In contrast, most colicins are at least 55-times larger than an average antibiotic, with colicins SR4 and E4 weighing ~61.4 and ~57.8 kDa, respectively. MICs reported in μg/mL show the lowest concentration (by weight) of a drug at which inhibition is observed, without taking into account how many molecules of drug are present. To avoid this potential MIC reporting bias, MICs are often reported in nanomoles (nM) [25,26].

MICs reported in nM (Table 5) reveal that far less colicin is required to inhibit the average isolate tested compared with gentamicin; colicins achieve the same antimicrobial activity as gentamicin using 20-30 times less drug.

4.2. Creation of a colicin-impregnated lubricant

The MIC of colicin SR4 against a CLSI *E. coli* standard strain (ATCC25922) is 2.85μg/mL (46 nM). This concentration was used in our experimental lubricant formulation.

4.2.1. Prevention of catheter contamination

The colicin SR4-impregnated lubricant was tested to see if it reduced or eliminated catheter contamination after exposure to

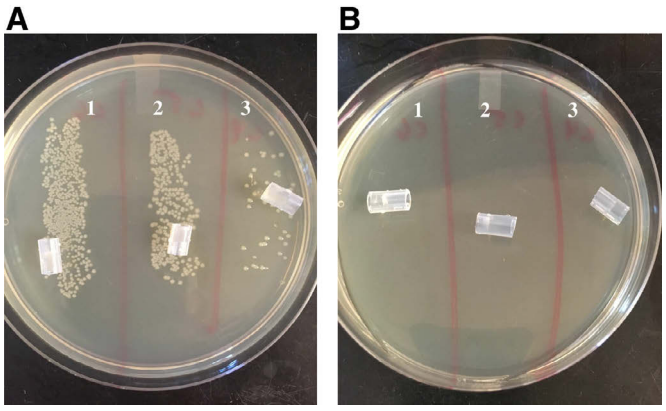

Fig. 2. Prevention of catheter contamination by (A) lubricant-only or (B) colicin-impregnated lubricant coated catheters incubated in Luria Broth containing 10^3 *E. coli* for 24 h.

E. coli. Catheter sections were lubricated with either the control (lubricant alone) or experimental formulation (lubricant and colicin SR4) and incubated in Luria Broth containing 10^3 cell/mL of *E. coli* strain ATCC25922. The catheter sections were removed after 10 min or 24 h. Catheters were left to drip dry and then rolled across an LB Agar petri plate. Figure 2 provides an illustration of these results. The lubricant alone plate (Figure 2A) reveals *E. coli* growth across the path of the catheter. In contrast, catheters treated with the colicin-impregnated lubricant show no *E. coli* growth (Figure 2B).

Cell densities of the Luria Broth were tested 10 min and 24 h post-incubation. The lubricant-only catheter had a slight decrease in cell density after 10 min (45% reduction), but exponential growth was observed after 24 h. The colicin-impregnated lubricant resulted in an 87% reduction in cell densities after 10 min and by 24 h no viable cells remained.

These data indicate that use of a colicin-containing lubricant during catheter insertion could result in complete elimination of colicin-sensitive *E. coli* contamination. Although colicin-resistant strains would not be eliminated, these data show that these are the strains most likely to be sensitive to conventional antibiotics, making treatment of any resulting CAUTI more feasible.

4.2.2. Stability

The stability of colicin SR4 and the colicin-impregnated lubricant formulation was assayed in different conditions of pH, time, and temperature. There was no impact of pH on *E. coli* inhibition in the tested range of pH 4 to 9. Colicin SR4 protein retained activity for over 2 years when stored at -80°C, for 6 months at -20°C, and more than a week at 40°C. The colicin SR4-impregnated lubricant maintained antimicrobial activity for over one month when stored at -20°C, 4°C, and 25°C, and lost activity at 37°C and 40°C after one week.

A sterile lubricant formulation for short-term catheterization is required to survive at 37°C for up to 30 days. Additional formulation efforts are clearly required, but data from other bacteriocins indicate this hurdle is not insurmountable. For example, nisin (a bacteriocin produced by *Lactococcus lactis*) is commercially available and has a documented shelf-life of two years [27]. Furthermore, the colicin-impregnated lubricant retains activity for a week or more at body temperature, thus supporting the promise of this novel approach to prevent extraluminal catheter contamination.

5. Conclusion

This paper describes a novel approach to prevent extraluminal urinary catheter contamination by *E. coli* using colicin proteins in a lubricant formulation to prevent or eliminate bacterial contamination. The data indicate the great promise of this approach. Colicins have been shown to be inhibitory against the target uropathogens, to lack collateral damage against members of the microbiome, to be stable under physiologically relevant conditions, and complementary in activity to currently used antibiotics.

The data presented here show that colicins can achieve the same killing activity as the antibiotic gentamicin at 10-fold lower concentrations. Considering the lack of colicin toxicity against human cells relative to antibiotics [26,28] and the fact that numerous colicins have achieved GRAS (generally recognized as safe) status from the US Food and Drug Administration (FDA), colicins will likely display promising pharmacokinetic and pharmacodynamic properties as they are further explored for use as alternatives, or complements, to existing antibiotics.

A colicin-impregnated lubricant is both feasible and practical, with no changes required to the catheter insertion protocol, only a substitution in lubricants; this may reduce the perceived risk threshold of healthcare providers in implementing this new drug. All these data reinforce the power of a narrow-spectrum approach to infection control and support the creation of a paradigm-shift in prophylaxis against catheter contamination.

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Competing interests: None.

Ethical approval: Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2019.07.004.

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