On the Etiological Relevance of *Escherichia coli* and *Staphylococcus aureus* in Superficial and Deep Infections – A Hypothesis-Forming, Retrospective Assessment

Hagen Frickmann[1,2*], Andreas Hahn[2], Stefan Berlec[2], Johannes Ulrich[2], Moritz Jansson[2], Norbert Georg Schwarz[3], Philipp Warnke[2] and Andreas Podbielski[2]

[1]*Department of Microbiology and Hospital Hygiene, Bundeswehr Hospital Hamburg, Hamburg, Germany*
[2]*Institute for Medical Microbiology, Virology, and Hygiene, University Medicine Rostock, Rostock, Germany*
[3]*Infectious Disease Epidemiology, Bernhard Nocht Institute for Tropical Medicine Hamburg, Hamburg, Germany*

Received: 26 Aug 2019; accepted: 08 Sep 2019

Introduction: *Escherichia coli* and *Staphylococcus aureus* are important causes of severe diseases like blood stream infections. This study comparatively assessed potential differences in their impact on disease severity in local and systemic infections.
Methods: Over a 5-year interval, patients in whom either *E. coli* or *S. aureus* was detected in superficial or primary sterile compartments were assessed for the primary endpoint death during hospital stay and the secondary endpoints duration of hospital stay and infectious disease as the main diagnosis.
Results: Significance was achieved for the impacts as follows: Superficial infection with *S. aureus* was associated with an odds ratio of 0.27 regarding the risk of death and of 1.42 regarding infectious disease as main diagnosis. Superficial infection with *E. coli* was associated with a reduced duration of hospital stay by −2.46 days and a reduced odds ratio of infectious diseases as main diagnosis of 0.04. The hospital stay of patients with *E. coli* was increased due to third-generation cephalosporin and ciprofloxacin resistance, and in the case of patients with *S. aureus* due to tetracycline and fusidic acid resistance.
Conclusions: Reduced disease severity of superficial infections due to both *E. coli* and *S. aureus* and resistance-driven prolonged stays in hospital were confirmed, while other outcome parameters were comparable.

Keywords: etiological relevance, infection, bacterium, virulence, resistance

1. Introduction

Staphylococcus aureus and *Escherichia coli* frequently cause superficial and systemic infections [1–10]. Further, both species are among the most frequent causes of bacteremia and sepsis in Western industrialized countries [11–16], with observed proportions of 16.3% to 21.6% for *S. aureus* and 5.6% to 24.2% for *E. coli* among all causes of sepsis, associated with considerable morbidity and mortality [17, 18]. Prolonged antibiotic therapy is recommended for *S. aureus*-associated bacteremia due to high risk of secondary foci of infection and particularly high mortality [19, 20], both for methicillin-susceptible and for methicillin-resistant strains [17]. Along with systemic infections, both species can play a role in superficial infections such as wound infections [21–23] or in urinary tract infections [24, 25].

While the etiological relevance of both of these facultatively pathogenic species under the conditions described can be considered as well documented, the pathogenic potential of individual strains depends on the presence or absence of pathogenic factors and toxins [26, 27] and may vary. The pathogenic factors of *E. coli* comprise adhesins (fimbrial as well as afimbrial ones and outer membrane proteins), curli, flagella, fimbriae, invasins, iron acquisition factors (siderophores), lipopolysaccharides, pili, polysaccharide capsules, secreted serine proteases, and metalloproteases, and toxins like oligopeptides, AB (alpha and beta subunit)-toxins, and RTX (repeats in toxin) pore-forming toxins [25, 26, 28–32]. For *S. aureus*, agglutinins, coagulases and staphylokinases, exoenzymes like nucleases and proteases, secreted toxins such as host protease modulators, pore-forming toxins, and superantigens, as well as the ability of forming biofilms due to cell surface-associated proteins, are among the described pathogenic factors [33–41]. As recently discussed elsewhere [42, 43], resistance against bactericidal first-line drugs like beta-lactam antibiotics, caused by enzymes like, e.g., extended spectrum beta-lactamases (ESBL) or carbapenems for *E. coli*, as well as by penicillin-binding proteins for methicillin-resistant *S. aureus* (MRSA), can become a severe problem for medical care.

However, previous studies have suggested a trade-off between resistance and pathogenicity [44, 45], assuming that expressing resistance determinants may cost additional energy and could thus decrease the fitness and competitiveness of the strain. Highly varying resistance rates have been observed in *S. aureus* and *E. coli* strains in previous assessments [2, 6, 7].

This study was conducted to provide hypotheses to be proven by future prospective studies, focusing on two questions. Firstly, we have performed a comparative head-to-head assessment of various systemic or superficial infections exclusively associated either with *S. aureus* or with *E. coli* to get hints on potentially differing disease severity as measured by the outcome parameters, namely, death during hospital stay, duration of hospital stay, and infectious disease as the main

*Author for correspondence: Department of Microbiology and Hospital Infection, Bundeswehr Hospital Hamburg, Bernhard Nocht Str. 74, 20359 Hamburg, Germany; E-mail: frickmann@bnitm.de; Tel: 0049-40-6947-28743; Fax: 0040-40-6947-28709.

DOI: 10.1556/1886.2019.00021

diagnosis. Secondly, associations of resistance with the clinical course of documented infections have been assessed to discern hints supporting the above-mentioned fitness cost hypothesis.

2. Patients and Methods

2.1. Study Design. The assessment was conducted as a single-center retrospective observational study over 5 years at a German university hospital. Inclusion criteria will be discussed in detail later under the respective heading. Data were obtained from a laboratory information system (LIS) of the DIN EN ISO 15189-accredited Institute for Medical Microbiology, Virology, and Hygiene of the University Medicine Rostock, Germany. In detail, cases were identified by screening for the search terms: *Staphylococcus aureus* and *Escherichia coli*.

For the identification of the bacterial isolates assessed in this study, VITEK 2 identification cards (bioMérieux, Marcy-l'Étoile, France) or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) with a Shimadzu/Kratos "AXIMA Assurance" MALDI-TOF mass spectrometer (Shimadzu Germany Ltd., Duisburg, Germany) were used. As described by the manufacturer (bioMérieux), alpha-cyano-4-hydroxycinnamic acid preparation was carried out for all strains prior to MALDI-TOF assessment. The spectral fingerprints obtained were interpreted using the Vitek MS-ID IVD-mode database version 3.2.0.-6. (bioMérieux). The equivalence of these methods regarding their diagnostic reliability has repeatedly been confirmed in the literature [46–49]. Antibiotic resistance was analyzed applying Clinical and Laboratory Standards Institute ((CLSI), CLSI M100-S17/M2-A9, M7-A7 January 2007; CLSI M100-S19, M2-A8, M7-A8 January 2009) and European Committee on Antimicrobial Susceptibility Testing ((EUCAST), Clinical Break Point Version 2 January 2012 and Version 3 January 2013) clinical breakpoints using the appropriate VITEK 2 AST cards in the course of the study. No adjustment for breakpoint changes was performed. Assessed antimicrobials comprised penicillins (ampicillin, ampicillin-sulbactam, oxacillin, and piperacillin–tazobactam), cephalosporins (3rd and 4th generation cephalosporins, as well as cefuroxime and cefoxitin), the carbapenem imipenem, fluoroquinolones (norfloxacin, ciprofloxacin, levofloxacin, and moxifloxacin), aminoglycosides (gentamicin and tobramycin), glycopeptides (vancomycin and teicoplanin), the macrolide erythromycin, the lincosamide clindamycin, the streptogramin quinupristin, tetracycline, the glycylcycline tigecycline, the drug combination co-trimoxazole, fosfomycin, fusidic acid, rifampicin, the oxazolidinone linezolid, the lipopeptide daptomycin, and mupirocin.

After the removal of copy strains, except for the first isolate of each case, inpatient cases were assessed anonymously. Each patient was counted only once. Data were anonymously extracted from the patients' case files and collected in Microsoft Excel worksheets for statistical analysis.

2.2. Outcome Parameters. Primary and secondary outcome parameters were defined, with death during hospital stay as the primary outcome parameter and the duration of hospital stay, as well as presence or absence of an infectious disease as main diagnosis (localized or systemic infections) versus a non-infectious disease-related main diagnosis (from the fields of cardiovascular diseases, endocrinology, gastroenterology, neurology, orthopedics and trauma surgery, pneumology, rheumatology and tumors, urology, and others) as secondary outcome parameters.

The outcome parameters were assessed for patients with infections due to either *S. aureus* or *E. coli* with additional focus on superficial and systemic infections. Superficial infections assessed comprised skin and urinary tract infections, while lower respiratory tract infections, bacteremia, and infections of primary sterile compartments were defined as systemic infections.

Association of antibiotic resistance with the assessed outcome-parameter was also investigated.

2.3. Factors Potentially Affecting the Outcome. In addition to the outcome parameters described above, a number of factors were documented to assess potential effects on the outcome parameters. These variables comprised continuous parameters, such as age, and also noncontinuous parameters, such as gender; isolation site of the strain; non-surgical ward vs. surgical and intensive care ward; main diagnoses from the fields of cardiovascular disease, endocrinology/metabolic disorders, gastroenterology, local infections or systemic infections, neurology, orthopedics/traumatology, pulmonology, rheumatology, neoplasia, urology and others; peak values of leukocytes; procalcitonin (PCT) and C-reactive protein (CRP); and the presence or absence of antibiotic treatment at the time of hospital admission. Leukocytes, CRP, and PCT were semi-quantitatively categorized as shown in Table 1. Sample materials in which *S. aureus* or *E. coli* were identified were grouped as abscess materials, ascites, aspirates, biopsies and invasive foreign material, blood cultures, bronchial lavage, respiratory secretions, wound swabs, and urine. Antibiotic susceptibility or resistance as defined by EUCAST was recorded for comparison within the species.

The distribution of both the outcome parameters and potentially confounding variables is presented in Table 2 for both *S. aureus* and *E. coli*.

2.4. Inclusion and Exclusion Criteria. Patients were included if either *S. aureus* or *E. coli* was identified in the microbiological laboratory in any clinical sample material and if clinical information from the case files was available.

Incompleteness of the assessable dataset alone was not considered as exclusion criterion, although it led to a reduction in the number of interpretable cases.

2.5. Statistical Assessment. Statistical assessment was done using STATA 15.1 (StataCorp, USA) with an exploratory aim. Binary logistic regression was used for the binary endpoint parameters, namely, death and infectious disease as main diagnosis. Linear regression was used for the endpoint parameter duration of hospital stay. All models were implemented as backward selection models with a significance level of 0.1 to exclude parameters from the model. Modeling was performed for the whole dataset (global modeling), as well as for patients with either *S. aureus* or *E. coli* (local modeling). Comparisons between disjoint subpopulations generally refer to their complement. As there have been no defined references in this study, for practical reasons, the category with the lowest score or the first

Table 1. Algorithm of semi-quantification of leukocyte counts as well as CRP and PCT concentrations

Parameter	Unit	Reference value	Category 1	Category 2	Category 3	Category 4
Leukocyte count	10^9/L	4–9	Reduced (<4)	Normal (4–9)	Increased (>9)	
CRP	mg/L	<5	Normal (<5)	Slightly increased (5–50)	Moderately increased (>50–100)	Severely increased (>100)
PCT	ng/L	<0.06	Normal (<0.06)	Slightly increased (>0.06–10)	Moderately increased (>10–100)	Severely increased (>100)

Table 2. Overview on the distribution of individually assessed parameters in patients with *S. aureus* or *E. coli* infections

Parameter	*S. aureus* $n = 1040$	*E. coli* $n = 975$	Overall $n = 2015$
Death *n* (%)	128 (12.4%)	91 (9.3%)	219 (10.9%)
Gender			
Male	648 (62.3%)	423 (43.4%)	1071 (53.2%)
Female	392 (37.7%)	552 (56.6%)	944 (46.9%)
Patient group			
Systemic infection	405 (38.9%)	353 (36.2%)	758 (37.6%)
Superficial infection	557 (53.6%)	482 (49.4%)	1039 (51.6%)
Combined superficial and systemic infection	78 (5.5%)	140 (14.4%)	218 (10.8%)
Sample materials			
Ascites	3 (0.3%)	10 (1.0%)	13 (0.7%)
Biopsies and invasive foreign material	47 (4.5%)	428 (43.9%)	475 (23.6%)
Blood cultures	234 (22.5%)	245 (25.1%)	479 (23.8%)
Bronchial lavage	17 (1.6%)	19 (2.0%)	36 (1.8%)
Respiratory secretions	127 (12.2%)	37 (3.8%)	164 (8.1%)
Aspirates	14 (1.4%)	35 (3.6%)	49 (2.4%)
Abscess materials	70 (6.7%)	73 (7.5%)	143 (7.1%)
Wound swabs	501 (48.2%)	27 (2.8%)	528 (26.2%)
Urine	27 (2.6%)	101 (10.4%)	128 (6.4%)
Main diagnoses			
Endocrinology/metabolic disorders	16 (1.5%)	4 (0.4%)	20 (1.0%)
Gastroenterology	56 (5.4%)	175 (17.8%)	231 (11.5%)
Cardiovascular disease	99 (9.5%)	82 (8.4%)	181 (9.0%)
Local infection	333 (32.0%)	35 (3.6%)	368 (18.3%)
Systemic infection	103 (9.9%)	183 (18.8%)	286 (14.2%)
Rheumatology and neoplasia	108 (10.4%)	131 (13.4%)	239 (11.7%)
Neurological disorder	116 (11.2%)	24 (2.5%)	140 (7.0%)
Orthopedics/Traumatology	79 (7.6%)	24 (2.4%)	103 (5.1%)
Pulmonary disease	60 (5.8%)	80 (8.2%)	140 (7.0%)
Other diseases	37 (3.6%)	50 (5.1%)	87 (4.3%)
Urologic disease	33 (3.2%)	187 (19.2%)	220 (10.9%)
Ward			
Surgical	422 (40.6%)	394 (40.4%)	816 (40.5%)
Medical	618 (59.4%)	581 (59.6%)	1199 (59.5%)
Previous antibiotic therapy			
Not documented	594 (57.1%)	665 (68.2%)	1259 (62.5%)
Documented	446 (42.9%)	310 (31.8%)	756 (37.5%)
Ampicillin			
Susceptible	40 (37.7%)	462 (47.7%)	502 (46.7%)
Resistant	66 (62.3%)	507 (52.3%)	573 (53.3%)
Levofloxacin			
Susceptible	841 (85.8%)	n.a.	841 (85.8%)
Resistant	139 (14.2%)	n.a.	139 (14.2%)
Norfloxacin			
Susceptible	220 (85.9%)	n.a.	220 (85.9%)
Resistant	36 (14.1%)	n.a.	36 (14.1%)
Ciprofloxacin			
Susceptible	221 (86.0%)	728 (75.2%)	949 (77.5%)
Resistant	36 (14.0%)	240 (24.8%)	276 (22.5%)
Moxifloxacin			
Susceptible	860 (87.8%)	n.a.	860 (87.8%)
Resistant	119 (12.2%)	n.a.	119 (12.2%)
Erythromycin			
Susceptible	880 (89.8%)	n.a.	880 (89.8%)
Resistant	100 (10.2%)	n.a.	100 (10.2%)
Clindamycin			
Susceptible	885 (90.3%)	n.a.	885 (90.3%)
Resistant	95 (9.7%)	n.a.	95 (9.7%)
Quinupristin			
Susceptible	256 (100%)	n.a.	256 (100%)
Resistant	0	n.a.	0
Gentamicin			
Susceptible	957 (97.8%)	915 (94.3%)	1872 (96.1%)
Resistant	22 (2.3%)	55 (5.7%)	77 (4.0%)
Tetracycline			
Susceptible	905 (92.4%)	390 (61.2%)	1295 (80.1%)
Resistant	74 (7.6%)	247 (38.8%)	321 (19.9%)
Tobramycin			
Susceptible	822 (97.4%)	n.a.	822 (97.4%)
Resistant	22 (2.6%)	n.a.	22 (2.6%)
Tigecycline			
Susceptible	722 (100%)	426 (100%)	1148 (100%)
Resistant	0	0	0
Co-trimoxazole			
Susceptible	972 (99.3%)	686 (70.4%)	1658 (84.9%)
Resistant	7 (0.7%)	289 (29.6%)	296 (15.2%)
Fosfomycin			
Susceptible	972 (99.4%)	334 (99.4%)	1306 (99.4%)
Resistant	6 (0.6%)	2 (0.6%)	8 (0.6%)
Fusidic acid			
Susceptible	950 (97.5%)	n.a.	950 (97.5%)
Resistant	24 (2.5%)	n.a.	24 (2.5%)
Rifampicin			
Susceptible	976 (99.7%)	n.a.	976 (99.7%)
Resistant	3 (0.3%)	n.a.	3 (0.3%)
Oxacillin			
Susceptible	1035 (100%)	n.a.	1035 (100%)
Resistant	0	n.a.	0
Ampicillin–Sulbactam			
Susceptible	948 (100%)	580 (65.2%)	1528 (83.1%)
Resistant	0	310 (84.8%)	310 (16.9%)
Cefoxitin			
Susceptible	964 (100%)	n.a.	964 (100%)
Resistant	0	n.a.	0
Cefuroxime			
Susceptible	948 (100%)	n.a.	948 (100%)
Resistant	0	n.a.	0
Imipenem			
Susceptible	948 (100%)	974 (100%)	1922 (100%)
Resistant	0	0	0
Teicoplanin			
Susceptible	978 (100%)	n.a.	978 (100%)
Resistant	0	n.a.	0
Vancomycin			
Susceptible	978 (100%)	n.a.	978 (100%)
Resistant	0	n.a.	0
Linezolid			
Susceptible	980 (100%)	n.a.	980 (100%)
Resistant	0	n.a.	0
Daptomycin			
Susceptible	137 (99.3%)	n.a.	137 (99.3%)
Resistant	1 (0.7%)	n.a.	1 (0.7%)
Mupirocin			
Susceptible	963 (99.8%)	n.a.	963 (99.8%)
Resistant	2 (0.2%)	n.a.	2 (0.2%)
Third-generation cephalosporins			
Susceptible	n.a.	835 (88.6%)	835 (88.6%)
Resistant	n.a.	108 (11.5%)	108 (11.5%)
Fourth-generation cephalosporins			
Susceptible	n.a.	901 (92.6%)	901 (92.6%)
Resistant	n.a.	72 (7.4%)	72 (7.4%)
Piperacillin–Tazobactam			
Susceptible	n.a.	747 (85.4%)	747 (85.4%)
Resistant	n.a.	128 (14.6%)	128 (14.6%)
Days in hospital (available for (*n*))	1440	974	2014
Mean (SD)	19.6 (19.2)	15.3 (16.3)	17.6 (18.0)
Median	14.0	10.0	12.0
Days to sample acquisition (available for (*n*))	1040	975	2015
Mean (SD)	4.7 (10.4)	3.8 (7.5)	4.3 (9.1)
Median	1.0	1.0	1.0
Age (available for (*n*))	1040	975	2015
Mean (SD)	58.3 (22.0)	69.3 (15.1)	63.6 (19.7)
Median	63.0	72.0	69.0
CRP (available for (*n*))	1009	899	1908
Mean (SD)	1.9 (1.1)	2.1 (1.0)	2.0 (1.0)
Median	2.0	2.0	2.0
PCT (available for (*n*))	242	157	399
Mean (SD)	1.2 (0.5)	1.3 (0.2)	1.3 (0.5)
Median	1.0	1.0	1.0
Leukocytes (available for (*n*))	998	975	1973
Mean (SD)	2.5 (0.6)	2.5 (0.6)	2.5 (0.6)
Median	3.0	3.0	3.0

Table 3. Explorative binary logistic regression model with backward selection for the global model with patients with *S. aureus* and/or *E. coli* infections. Association with the outcome parameter death

Association with the outcome parameter death	Global modeling with both patients with *S. aureus* and patients with *E. coli*		Modeling with patients with *S. aureus*		Modeling with patients with *E. coli*	
	Odds ratio with 0.95 CI	P value	Odds ratio with 0.95 CI	P value	Odds ratio with 0.95 CI	P value
Isolation from biopsies and invasive foreign material	0.52 (0.32, 0.85)	0.009	n.a.	n.a.	0.09 (0.04, 0.29)	<0.001
Isolation from blood cultures	0.71 (0.47, 1.06)	0.091	n.a.	n.a.	0.13 (0.06, 0.29)	<0.001
Isolation from respiratory secretions	n.a.	n.a.	n.a.	n.a.	0.31 (0.09, 1.11)	0.073
Isolation from aspirates	n.a.	n.a.	n.a.	n.a.	0.26 (0.07, 1.00)	0.050
Isolation from abscess materials	2.34 (1.47, 3.72)	<0.001	n.a.	n.a.	n.a.	n.a.
Isolation from urine	n.a.	n.a.	n.a.	n.a.	0.21 (0.08, 0.56)	0.002
Gastroenterology	0.60 (0.36, 1.00)	0.051	n.a.	n.a.	n.a.	n.a.
Cardiovascular disease	n.a.	n.a.	n.a.	n.a.	2.33 (1.00, 5.47)	0.050
Local infection	0.61 (0.40, 0.91)	0.016	n.a.	n.a.	n.a.	n.a.
Rheumatology and neoplasia	0.56 (0.32, 0.97)	0.039	n.a.	n.a.	n.a.	n.a.
Pulmonary disease	n.a.	n.a.	n.a.	n.a.	2.28 (1.04, 4.97)	0.039
Urologic disease	0.04 (0.01, 0.30)	0.002	n.a.	n.a.	0.12 (0.02, 0.90)	0.039
Documented previous antibiotic therapy	1.39 (1.02, 1.89)	0.037	n.a.	n.a.	1.78 (0.99, 3.20)	0.055
Leukocyte count	0.79 (0.62, 1.01)	0.063	n.a.	n.a.	n.a.	n.a.
Age	n.a.	n.a.	n.a.	n.a.	1.03 (1.00, 1.05)	0.019
Superficial infections	n.a.	n.a.	0.27 (0.17, 0.41)	<0.001	n.a.	n.a.
Clindamycin resistance	n.a.	n.a.	1.73 (0.93, 3.22)	0.083	n.a.	n.a.
	N 1883, Pseudo R^2 0.0684		*N* 851, Pseudo R^2 0.0604		*N* 745, Pseudo R^2 0.1901	

Odds ratios >1 indicate a risk association with the outcome "death," but odds ratios <1 indicate a protective association.

Table 4. Explorative linear regression model with backward selection for the global model with patients with *S. aureus* and/or *E. coli* infections. Association with the outcome parameter duration of hospital stay

Association with the outcome parameter duration of hospital stay	Global modeling with both patients with *S. aureus* and patients with *E. coli*		Modeling with patients with *S. aureus*		Modeling with patients with *E. coli*	
	Odds ratio with 0.95 CI	P value	Odds ratio with 0.95 CI	P value	Odds ratio with 0.95 CI	P value
Biopsies and invasive foreign material	−1.36 (−2.23, −0.49)	0.002	n.a.	n.a.	−2.00 (−3.14, −0.88)	0.001
Blood cultures	n.a.	n.a.	1.40 (0.08, 2.71)	0.037	−3.15 (−4.49, −1.81)	<0.001
Bronchial lavage	n.a.	n.a.	n.a.	n.a.	−5.51 (−8.45, −2.56)	<0.001
Respiratory secretions	n.a.	n.a.	2.16 (0.52, 3.80)	0.010	−2.89 (−5.48, −0.29)	0.029
Aspirates	n.a.	n.a.	n.a.	n.a.	−2.55 (−4.66, −0.44)	0.018
Wound swabs	−1.16 (−1.98, −0.34)	0.005	n.a.	n.a.	n.a.	n.a.
Gastroenterology	3.18 (2.11, 4.25)	<0.001	5.18 (2.84, 7.52)	<0.001	2.57 (1.36, 3.79)	<0.001
Cardiovascular disease	1.68 (0.50, 2.85)	0.005	n.a.	n.a.	2.26 (0.70, 3.81)	0.005
Rheumatology and neoplasia	2.90 (1.79, 4.01)	<0.001	2.98 (1.01, 4.06)	0.003	2.86 (1.54, 4.17)	<0.001
Pulmonary disease	2.01 (0.72, 3.30)	0.002	n.a.	n.a.	2.83 (1.27, 4.38)	<0.001
Other diseases	2.79 (1.14, 4.43)	0.001	3.15 (0.19, 6.19)	0.037	2.39 (0.45, 4.32)	0.016
Urologic disease	1.99 (0.85, 3.13)	0.001	n.a.	n.a.	2.18 (0.99, 3.38)	<0.001
Previous antibiotic therapy	1.46 (0.79, 2.13)	<0.001	2.10 (1.01, 3.20)	<0.001	n.a.	n.a.
CRP	−0.47 (−0.80, −0.14)	0.005	−0.73 (−1.28, −0.18)	0.010	−0.44 (−0.83, −0.04)	0.032
Age	−0.036 (−0.05, −0.02)	<0.001	−0.04 (−0.06, −0.01)	0.002	n.a.	n.a.
Third-generation cephalosporin resistance	n.a.	n.a.	n.a.	n.a.	2.59 (1.38, 3.81)	<0.001
Tetracycline resistance	n.a.	n.a.	2.22 (0.25, 4.18)	0.027	n.a.	n.a.
Fusidic acid resistance	n.a.	n.a.	5.67 (2.26, 9.08)	0.001	n.a.	n.a.
Ciprofloxacin resistance	n.a.	n.a.	n.a.	n.a.	0.81 (−0.15, 1.76)	0.097
Superficial infection	n.a.	n.a.	n.a.	n.a.	−2.46 (−3.71, −1.21)	<0.001
	N 1883, adjusted R^2 0.3987		*N* 918, adjusted R^2 0.3863		*N* 774, adjusted R^2 0.5340	

Coefficients >0 indicate a prolonging association with the endpoint days to discharge (parameter extends on average by the shown number of days), coefficients <0 indicate a shortening association (parameter reduces on average by the shown number of days).

appearance in each group has been used as a reference for the calculations. Parameters with insufficient numbers for regression analysis were excluded from the modeling. For the endpoint-parameter infectious disease as a main diagnosis, the parameters of main diagnoses were not included into the model due to lack of independence.

2.6. Ethics. Ethical clearance for the assessment was obtained from the Ethics Committee of the University Medicine Rostock (Registration number A 2014–0054). The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Do to the retrospective design of the study, the assessment was allowed by the ethics committee in an anonymous way without consent to participate.

3. Results

3.1. Study Population. After removal of copy strains, the study population comprised 2015 cases: 1040 isolations of *S. aureus* and 975 isolations of *E. coli*. These isolations were associated with systemic infections in 758 cases, with superficial infections in 1039 cases, and with combined systemic and superficial infections in 218 cases.

Table 5. Explorative binary logistic regression model with backward selection for the global model with patients with *S. aureus* and/or *E. coli* infections. Association with the outcome parameter infectious disease as main diagnosis

Association with the outcome parameter infectious disease as main diagnosis	Global modeling with both patients with *S. aureus* and patients with *E. coli*		Modeling with patients with *S. aureus*		Modeling with patients with *E. coli*	
	Odds ratio with 0.95 CI	P value	Odds ratio with 0.95 CI	P value	Odds ratio with 0.95 CI	P value
Biopsies and invasive foreign material	n.a.	n.a.	n.a.	n.a.	4.26 (1.78, 10.19)	0.001
Blood cultures	5.53 (4.15, 7.38)	<0.001	n.a.	n.a.	5.69 (2.78, 11.68)	<0.001
Bronchial lavage	n.a.	n.a.	0.23 (0.05, 1.07)	0.061	n.a.	n.a.
Respiratory secretions	n.a.	n.a.	0.20 (0.11, 0.37)	<0.001	n.a.	n.a.
Aspirates	5.40 (2.92, 10.01)	<0.001	n.a.	n.a.	14.19 (5.24, 38.40)	<0.001
Abscess materials	5.52 (3.61, 8.46)	<0.001	4.88 (2.58, 9.25)	<0.001	n.a.	n.a.
Wound swabs	8.08 (6.06, 10.77)	<0.001	1.83 (1.33, 2.51)	<0.001	651.55 (157.87, 2689.04)	<0.001
Urine	n.a.	n.a.	0.13 (0.03, 0.60)	0.008	18.99 (6.41, 56.22)	<0.001
Age	0.99 (0.98, 0.99)	<0.001	0.99 (0.98, 0.99)	0.001	1.01 (1.00, 1.03)	0.083
Gender	1.25 (1.00, 1.54)	0.045	n.a.	n.a.	n.a.	n.a.
Leukocyte count	1.47 (1.22, 1.75)	<0.001	1.35 (1.06, 1.73)	0.016	1.71 (1.22, 2.41)	0.002
CRP	n.a.	n.a.	0.86 (0.74, 0.99)	0.034	1.43 (1.12, 1.81)	0.004
Superficial infections	n.a.	n.a.	1.42 (1.08, 1.87)	0.013	0.04 (0.02, 0.08)	<0.001
Surgical and intensive care wards	n.a.	n.a.	n.a.	n.a.	0.18 (0.11, 0.30)	<0.001
	N 1885, Pseudo R^2 0.1385		*N* 986, Pseudo R^2 0.1292		*N* 899, Pseudo R^2 0.3473,	

Odds ratios >1 indicate a risk association with the outcome infectious disease as main diagnosis, whereas odds ratios <1 indicate a protective association.

3.2. Assessed Parameters. Detailed information on the distribution of assessed patient and strain characteristics is provided in Table 2. Thereby, parameters are presented as distributed by either species of the bacterial isolates (*S. aureus* or *E. coli*), as well as for the whole assessed population. Based on those data, global modeling for the whole dataset, as well as local modeling for patients with either *S. aureus* or *E. coli* with a focus on the study endpoints, was performed; the results are presented in the following sections.

3.3. Assessment of the Study Endpoints. Neither isolation of *S. aureus* nor that of *E. coli* was associated with any of the endpoint parameters in the global modeling. However, superficial infection with *S. aureus* was negatively associated with the primary outcome parameter death ($P < 0.001$), but it was positively associated with infectious disease as the main diagnosis ($P = 0.013$). Superficial infection with *E. coli*, in contrast, was associated with shorter duration of hospital stay ($P < 0.001$) and negatively associated with infectious disease as main diagnosis ($P < 0.001$). Several variables potentially affected the primary and secondary outcome parameters as shown in Tables 3–5.

3.4. Associations between Resistance and Superficial or Systemic Infections. Enhanced resistance, i.e., resistance to 3 or more antibiotic substance classes, was not generally positively or negatively associated with invasive infections.

Resistance against tetracycline ($P = 0.027$) and fusidic acid ($P < 0.001$) in patients with *S. aureus* and against third-generation cephalosporins ($P < 0.001$) and ciprofloxacin ($P = 0.097$) in patients with *E. coli* was associated with increased duration of hospital stay. Also, there was a tendency for an increased risk of death in patients with clindamycin-resistant *S. aureus* ($P = 0.083$) (Tables 3 and 4).

4. Discussion

The study was conducted to analyze any differences in the etiological relevance of *S. aureus* and *E. coli* over a study period of 5 years with inpatients at a German university hospital with superficial or systemic infections. The focus was on the primary endpoint death during hospital stay, and the two secondary endpoints, duration of hospital stay and a main diagnosis of infectious disease. Potential associations between resistance and severity of the infectious diseases were also assessed.

The results of the assessment differed for the different cases. First of all, neither *S. aureus* nor *E. coli* as individual species alone were associated with any of the outcome parameters. As expected, superficial infections with *S. aureus* were associated with reduced risk of death compared with systemic infections; most interestingly, this association was not seen for superficial *E. coli* infections. The high relevance of *E. coli* for urinary tract infections [30] (which were classed in the superficial infection group in this study) and their specific complications is a likely reason for this. Pathogenic factors associated with uropathogenic *E. coli* comprise fimbriae, curli, pili, capsules, iron scavenger receptors, flagella, toxins, and lipopolysaccharides [25, 26, 30]. In contrast, superficial *E. coli* infections were associated with a decrease of duration of hospital stay and the likelihood of infectious disease as the main diagnosis compared with systemic infections. Also in contrast, the prominence of *S. aureus*-associated skin and soft-tissue infections [50] allowed an association of superficial *S. aureus* infections and infectious disease as main diagnosis. Clumping factor B, Panton–Valentine leukocidin, and bi-component pore-forming toxins are prominent virulence factors that have been associated with skin and soft tissue infections due to *S. aureus* [51–54]. Admittedly, molecular screening for virulence factors was beyond the scope of this study.

Antibiotic drug resistance was only weakly associated with disease severity as measured by the chosen outcome parameters. Potential resistance factors associated with the observed disease severity-associated resistance patterns comprise ribosomal methylases or efflux pumps causing clindamycin resistance [55], tetracycline resistance genes of the *tet* gene family associated with tetracycline resistance [56], or fusidic acid resistance genes of the *fus* gen family associated with fusidic acid resistance [57] in *S. aureus*, as well as beta-lactamases associated with 3rd generation cephalosporin resistance in *E. coli* [55]. In all observed cases, higher levels of resistance were associated with increased disease severity. Accordingly, this hypothesis-forming assessment provided no indications supporting the trade-off theory of fitness cost and antibiotic resistance, as suggested in the literature [44, 45]. Admittedly, the study was only an explorative assessment, and pathogenic factors of the isolates were not assessed, an undeniable limitation of the study. Further, applied interpretation standards of resistance testing have been changed in the course of the

study, which interferes with interpretability, although major discrepancies are nevertheless unlikely.

The study has a number of further limitations. Since the study is a retrospective exploration, conclusions regarding associative relationships can only provide hypotheses. In addition, especially in the global model of both groups with either *S. aureus* or *E. coli* and in the local model for patients with *S. aureus*, the models account for only a small part of the total dispersion, so it must be assumed that a considerable amount of information that could have been explanatory was not collected. However, the hypotheses derived from this study can be used to guide future prospective studies.

5. Conclusion

In conclusion, the study did not show specific association of disease severity, as defined by the endpoint parameters with the isolation of either *S. aureus or E. coli*, while, as expected, superficial infections were generally associated with milder diseases in comparison to systemic infections, as suggested by the outcome parameters. Interestingly, a reduced risk of death was shown for superficial infections due to *S. aureus* but not for those due *to E. coli*, in comparison to systemic infections. As far as can be determined despite the limitations of the study, resistance was associated with increased disease severity as defined by the endpoint parameters, so the phenomenon of trade-off between resistance and pathogenicity was not supported.

Funding Sources

No financial support was received for this study.

Authors' Contributions

JU, SB, and UJ conducted the collection and assessments of the data. AP and PW performed the laboratory assessments. AH and NGS were in charge of the statistical assessment. AH, AP, HF, and PW planned the design of the retrospective study. All authors jointly wrote and optimized the manuscript.

Conflict of Interest

Nothing to declare.

References

1. Armed Forces Health Surveillance Center (AFHSC). Septicemia diagnosed during hospitalizations, active component service members, U.S. Armed Forces, 2000–2012. MSMR. 2013;20:10–6.

2. Sherwood J, Park M, Robben P, Whitman T, Ellis MW. USA300 methicillin-resistant *Staphylococcus aureus* emerging as a cause of bloodstream infections at military medical centers. Infect Control Hosp Epidemiol. 2013;34:393–99.

3. Landrum ML, Neumann C, Cook C, Chukwuma U, Ellis MW, Hospenthal DR, et al. Epidemiology of *Staphylococcus aureus* blood and skin and soft tissue infections in the US military health system, 2005-2010. JAMA. 2012;308:50–9.

4. Ressner RA, Murray CK, Griffith ME, Rasnake MS, Hospenthal DR, Wolf SE. Outcomes of bacteremia in burn patients involved in combat operations overseas. J Am Coll Surg. 2008;206:439–44.

5. Weintrob AC, Murray CK, Xu J, Krauss M, Bradley W, Warkentien TE, et al. Early Infections Complicating the Care of Combat Casualties from Iraq and Afghanistan. Surg Infect. 2018;19:286–97.

6. Campbell WR, Li P, Whitman TJ, Blyth DM, Schnaubelt ER, Mende K, et al. Multi-Drug-Resistant Gram-Negative Infections in Deployment-Related Trauma Patients. Surg Infect. 2017;18:357–67.

7. Mende K, Beckius ML, Zera WC, Onmus-Leone F, Murray CK, Tribble DR. Low Prevalence of carbapenem-resistant *Enterobacteriaceae* among wounded military personnel. US Army Med Dep J. 2017;2–17:12–7.

8. Mende K, Beckius ML, Zera WC, Yu X, Cheatle KA, Aggarwal D, et al. Phenotypic and genotypic changes over time and across facilities of serial colonizing and infecting *Escherichia coli* isolates recovered from injured service members. J Clin Microbiol. 2014;52:3869–77.

9. Fisher A, Webber BJ, Pawlak MT, Johnston L, Tchandja JB, Yun H. Epidemiology, microbiology, and antibiotic susceptibility patterns of skin and soft tissue infections, Joint Base San Antonio-Lackland, Texas, 2012-2014. MSMR. 2015;22:2–6.

10. Lamb L, Morgan M. Skin and soft tissue infections in the military. J R Army Med Corps. 2013;159:215–23.

11. Geerdes HF, Ziegler D, Lode H, Hund M, Loehr A, Fangmann W, Wagner J. Septicemia in 980 patients at a university hospital in Berlin: prospective studies during 4 selected years between 1979 and 1989. Clin Infect Dis. 1992;15:991–1002.

12. Rosenthal EJ. Septicemia causative organisms 1983-1985. The results of a multicenter study. Dtsch Med Wochenschr. 1986;111:1874–80.

13. Rosenthal EJ. Epidemiology of septicaemia pathogens. Dtsch Med Wochenschr. 2002;127:2435–40.

14. Rosenthal EJ. The epidemiology of septicemia causative agents. A blood culture study of the Paul Ehrlich Society for Chemotherapy e. V. Dtsch Med Wochenschr. 1993;118:1269–75.

15. Schaberg DR, Culver DH, Gaynes RP. Major trends in the microbial etiology of nosocomial infection. Am J Med. 1991;91(3B):72S–5S.

16. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis. 2004:39:309–17.

17. Yaw LK, Robinson JO, Ho KM. A comparison of long-term outcomes after meticillin-resistant and meticillin-sensitive *Staphylococcus aureus* bacteraemia: an observational cohort study. Lancet Infect Dis. 2014;14:967–75.

18. Bhattacharya A, Nsonwu O, Johnson AP, Hope R. Estimating the incidence and 30-day all-cause mortality rate of *Escherichia coli* bacteraemia in England by 2020/21. J Hosp Infect. 2018;98:228–31.

19. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med. 2017;43:304–77.

20. Jung N, Rieg S. Essentials in the management of *S. aureus* bloodstream infection. Infection. 2018;46:441–2.

21. Azzopardi EA, Azzopardi E, Camilleri L, Villapalos J, Boyce DE, Dziewulski P, et al. Gram negative wound infection in hospitalised adult burn patients–systematic review and metanalysis. PLoS One. 2014;9:e95042.

22. Bessa LJ, Fazii P, Di Giulio M, Cellini L. Bacterial isolates from infected wounds and their antibiotic susceptibility pattern: some remarks about wound infection. Int Wound J. 2015;12:47–52.

23. Serra R, Grande R, Butrico L, Rossi A, Settimio UF, Caroleo B, et al. Chronic wound infections: the role of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Expert Rev Anti Infect Ther. 2015;13:605–13.

24. Karakonstantis S, Kalemaki D. Evaluation and management of *Staphylococcus aureus* bacteriuria: an updated review. Infection. 2018;46:293–301.

25. Terlizzi ME, Gribaudo G, Maffei ME. UroPathogenic *Escherichia coli* (UPEC) Infections: Virulence Factors, Bladder Responses, Antibiotic, and Non-antibiotic Antimicrobial Strategies. Front Microbiol. 2017;8:566.

26. Lüthje P, Brauner A. Virulence factors of uropathogenic *E. coli* and their interaction with the host. Adv Microb Physiol. 2014;65:337–72.

27. Otto M. *Staphylococcus aureus* toxins. Curr Opin Microbiol. 2014;17:32–7.

28. Tapader R, Basu S, Pal A. A regulatory trade-off as a source of strain variation in the species *Escherichia coli*. J Bacteriol. 2004:86:5614–20.

29. Sarowska J, Futoma-Koloch B, Jama-Kmiecik A, Frej-Madrzak M, Ksiazczyk M, Bugla-Ploskonska G, et al. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: recent reports. Gut Pathog. 2019;11:10.

30. Asadi Karam MR, Habibi M, Bouzari S. Urinary tract infection: Pathogenicity, antibiotic resistance and development of effective vaccines against Uropathogenic *Escherichia coli*. Mol Immunol. 2019;108:56–67.

31. Johnson JR, Russo TA. Molecular Epidemiology of Extraintestinal Pathogenic *Escherichia coli*. EcoSal Plus. 2018;8:1.

32. Mainil J. *Escherichia coli* virulence factors. Vet Immunol Immunopathol. 2013;152:2–12.

33. Tam K, Torres VJ. *Staphylococcus aureus* Secreted Toxins and Extracellular Enzymes. Microbiol Spectr. 2019;7:2.

34. Singh V, Phukan UJ. Interaction of host and *Staphylococcus aureus* protease-system regulates virulence and pathogenicity. Med Microbiol Immunol. 2018, [Epub ahead of print]:doi: 10.1007/s00430-018-0573-y.

35. Oliveira D, Borges A, Simões M. *Staphylococcus aureus* Toxins and Their Molecular Activity in Infectious Diseases. Toxins, 2018;10:6.

36. Hecker M, Mäder U, Völker U. From the genome sequence via the proteome to cell physiology – Pathoproteomics and pathophysiology of *Staphylococcus aureus*. Int J Med Microbiol. 2018;308:545–57.

37. Dayan GH, Mohamed N, Scully IL, Cooper D, Begier E, Eiden J, et al. *Staphylococcus aureus*: the current state of disease, pathophysiology and strategies for prevention. Expert Rev Vaccines. 2016;15:1373–92.

38. Thomer L, Schneewind O, Missiakas D. Pathogenesis of *Staphylococcus aureus* Bloodstream Infections. Annu Rev Pathol. 2016;11:343–64.

39. Becker RE, Bubeck Wardenburg J. *Staphylococcus aureus* and the skin: a longstanding and complex interaction. Skinmed. 2015;13:111–9.

40. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015;28:603–61.

41. Powers ME, Bubeck Wardenburg J. Igniting the fire: Staphylococcus aureus virulence factors in the pathogenesis of sepsis. PLoS Pathog. 2014;10: e1003871.

42. Frickmann H, Podbielski A, Kreikemeyer B. Resistant Gram-Negative Bacteria and Diagnostic Point-of-Care Options for the Field Setting during Military Operations. Biomed Res Int. 2018;2018:9395420.

43. Frickmann H. Impact of MRSA on the Military Medical Service and Diagnostic Point-of-Care Options for the Field Setting. Eur J Microbiol Immunol. 2018;8:31–3.

44. King T, Ishihama A, Kori A, Ferenci T. A regulatory trade-off as a source of strain variation in the species *Escherichia coli*. J Bacteriol. 2004;186:5614–20.

45. Ferenci T. What is driving the acquisition of *mutS* and *rpoS* polymorphisms in *Escherichia coli*? Trends Microbiol. 2003;11:457–61.

46. Jo SJ, Park KG, Han K, Park DJ, Park YJ (2016) Direct identification and antimicrobial susceptibility testing of bacteria from positive blood culture bottles by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry and the Vitek 2 system. Ann Lab Med. 2016;36:117–23.

47. Angeletti S. Matrix assisted laser desorption time of flight mass spectrometry (MALDI-TOF-MS) in clinical microbiology. J Microbiol Meth. 2017;138:20–9.

48. Kassim A, Pflüger V, Preemji Z, Daubenberger C, Revathi G. Comparison of biomarker based Matrix Assisted Laser Desorption Ionization-Time od Flight Mass Spectrometry (MALDI-TOF-MS) and conventional methods in the identification of clinically relevant bacteria and yeast. BMC Microbiology. 2017;17:128.

49. Trevisoli LE, Bail L, Rodrigues LS, Conte D, Palmeiro JK, Dalla-Costa LM. Matrix-assisted laser desorption ionization-time of flight: a promising alternative method of identifying the major coagulase-negative Staphylococci species. Rev Soc Bras Med Trop. 2018;51:85–7.

50. Poulakou G, Lagou S, Tsiodras S. What's new in the epidemiology of skin and soft tissue infections in 2018? Curr Opin Infect Dis. 2019;32:77–86.

51. Lacey KA, Mulcahy ME, Towell AM, Geoghegan JA, McLoughlin RM. Clumping factor B is an important virulence factor during *Staphylococcus aureus* skin infection and a promising vaccine target. PLoS Pathog 2019;15: e1007713.

52. Klein S, Menz MD, Zanger P, Heeg K, Nurjadi D. Increase in the prevalence of Panton-Valentine leukocidin and clonal shift in community-onset methicillin-resistant *Staphylococcus aureus* causing skin and soft-tissue infections in the Rhine-Neckar Region, Germany, 2012-2016. Int J Antimicrob Agents. 2019;53:261–7.

53. Perumal N, Dass BS, Mani S, Krishnan P. Prevalence of bi-component pore-forming toxin genotypes of *Staphylococcus aureus* causing skin and soft tissue infections. Indian J Med Microbiol. 2017;35:146–7.

54. Jahamy H, Ganga R, Al Raiy B, Shemes S, Nagappan V, Sharma M, Riederer K, Khatib R. *Staphylococcus aureus* skin/soft-tissue infections: the impact of SCCmec type and Panton-Valentine leukocidin. Scand J Infect Dis. 2008;40(8):601–6.

55. Leclercq R, Cantón R, Brown DF, Giske CG, Heisig P, MacGowan AP, Mouton JW, Nordmann P, Rodloff AC, Rossolini GM, Soussy CJ, Steinbakk M, Winstanley TG, Kahlmeter G. EUCAST expert rules in antimicrobial susceptibility testing. Clin Microbiol Infect. 2013;19:141–60.

56. Schwarz S, Cardoso M, Wegener HC. Nucleotide sequence and phylogeny of the *tet(L)* tetracycline resistance determinant encoded by plasmid pSTE1 from *Staphylococcus hyicus*. Antimicrob Agents Chemother. 1992;36:580–8.

57. Chen HJ, Hung WC, Lin YT, Tsai JC, Chiu HC, Hsueh PR, Teng LJ. A novel fusidic acid resistance determinant, *fusF*, in *Staphylococcus cohnii*. J Antimicrob Chemother. 2015;70:416–9.