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High mortality risk of type III monomicrobial gram-negative necrotizing fasciitis: The role of extraintestinal pathogenic *Escherichia coli* (ExPEC) and *Klebsiella pneumoniae*

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ABSTRACT

Objectives: The aim of this study was to investigate the prognostic value of reclassified new type III monomicrobial gram-negative necrotizing fasciitis (NF) and the microbial factors associated with an increased risk of mortality.

Methods: This study included 235 NF cases treated at National Taiwan University Hospital. We compared the mortality risk of NF caused by different causal microorganisms and examined the bacterial virulence genes profile and antimicrobial susceptibility pattern associated with an increase in mortality risk.

Results: Type III NF (n = 68) had a mortality risk two-fold higher than type I (polymicrobial, n = 64) or type II (monomicrobial gram-positive, n = 79) NF (42.6% vs 23.4% or 19.0%, $P = 0.019$ and 0.002, respectively). Mortality differed by causal microorganism (*Escherichia coli* [61.5%], *Klebsiella pneumoniae* [40.0%], *Aeromonas hydrophila* [37.5%], *Vibrio vulnificus* [25.0%], polymicrobial [23.4%], group A streptococci [16.7%], and *Staphylococcus aureus* [16.2%], in decreasing rank, $P < 0.001$). Type III NF caused by *E. coli*, identified as extraintestinal pathogenic *E. coli* (ExPEC) via virulence gene analyses, was associated with a particularly high mortality risk (adjusted odds ratio: 6.51, $P = 0.003$) after adjusting for age and comorbidities. Some (38.5%/7.7%) of the *E. coli* strains were non-susceptible to third/fourth-generation cephalosporins but remained susceptible to carbapenems.

Conclusion: Type III NF, especially cases caused by *E. coli* or *K. pneumoniae*, are associated with a comparatively higher mortality risk than type I or type II NF. Wound gram stain-based rapid diagnosis of type III NF may inform empirical antimicrobial therapy to include a carbapenem.

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Introduction

Necrotizing fasciitis (NF) is a rapidly progressive soft tissue infection characterized by fascia plane spread and extensive tissue necrosis, which requires prompt surgical debridement [1,2]. NF is a life-threatening disease with a case fatality rate of 15–30%, which is affected by old age, underlying diseases, immunocompromising conditions, and the severity of NF [1]. In addition to timely surgical debridement [2], optimized antimicrobial therapy is crucial in reducing the mortality risk associated with NF [1,3].

Microbiologically, NF is classified into three types: type I is a polymicrobial infection involving mixed aerobic/anaerobic bacteria; type II is a monomicrobial infection caused by group A streptococci (GAS) or *Staphylococcus aureus*; and type III is caused by *Vibrio* species or *Aeromonas* species and is predominantly found

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in warm coastal regions [1,3–7]. Monomicrobial NF caused by *Escherichia coli* or *Klebsiella pneumoniae*, which is not included in the current NF classification, has been increasingly reported in Taiwan [8], South Korea [9], and Israel [10], accounting for up to 23.9% [8], 22.4% [11], and 22.2% [12] of all monomicrobial NF cases, respectively. In 2019, Kuehl *et al.* proposed an adapted classification for NF that includes all monomicrobial gram-negative NF cases as type III, based on their high mortality risk (especially in cases caused by Enterobacteriales) compared with that of type I or type II NF [9,13]. Monomicrobial gram-negative NF is more likely to occur in elderly patients with liver cirrhosis, chronic kidney diseases, or other comorbidities that are associated with poor outcomes [9,13].

Despite the increasing awareness, there is a lack of data regarding the role of microbial factors in the high mortality risk of type III necrotizing fasciitis. In Taiwan, gram-negative bacteria, including *K. pneumoniae, E. coli, V. vulnificus*, and *A. hydrophila*, are responsible for 40–50% of monomicrobial NF cases [8,11]. Hence, this study aimed to investigate the prognostic value of reclassified new type III (monomicrobial gram-negative) NF [13], as well as the bacterial virulence gene profile and antimicrobial susceptibility pattern associated with an increased risk of mortality in NF.

Methods

Study design

National Taiwan University Hospital (NTUH) is a 2200-bed university medical center that offers both primary and referral care in Taipei, Taiwan. The hospital provides services to approximately 3 million outpatients and 90,000 inpatients each year. We identified all patients with surgically confirmed NF treated at NTUH between September 1998 and September 2018, using the NF registry, NTUH Division of Plastic Surgery [8]. The clinical information was retrieved from the medical records using a computerized data collection form. We compared mortality risk of NF caused by different causal microorganisms and examined the bacterial virulence genes profile and antimicrobial susceptibility pattern associated with the increase in mortality risk.

Ethical statement

The study protocol (201804082RINA) was reviewed and approved by NTUH Research Ethics Committee and the need for informed consent was waived.

Inclusion and exclusion criteria

To be included as a surgically confirmed case of NF, both surgical and histopathological reports must indicate NF, such as "necrotic fascia and purulent malodorous discharge" [8], and "extensive tissue destruction, thrombosis of blood vessels, abundant bacteria spreading along fascial planes, and infiltration of acute inflammatory cells," respectively [9,12]. NF must be the principal diagnosis and confirmed independently by two investigators (N.-C. C. and C.-T. F.). We excluded cases in which the NF diagnosis was not surgically confirmed.

Identification of causal microorganism

Bacterial isolation, species identification, and antimicrobial susceptibility testing were routinely performed at NTUH in accordance with the United States National Committee for Clinical Laboratory Standards [14]. Two investigators (N.-C. C. and C.-T. F.) reviewed the medical records and microbiological reports. For each confirmed NF case, identification of the causal microorganism(s) was based on results of blood or surgical wound cultures obtained at the time of initial evaluation for NF (at the Emergency Department for community-acquired NF or Inpatient Services for hospital-acquired NF) or at the first surgery, as independently verified by both investigators.

Data collection

Two investigators (N.-C. C. and Y. C.) reviewed the medical records and systematically collected demographic and clinical data, including presentations (symptoms, signs, and laboratory results at initial evaluation for NF), comorbidities, time from symptoms onset to surgery (within 12 hours or more than 12 hours) [2], hospitalization duration, and outcomes. All patients were followed up to discharge from the hospital. Information was systematically coded using predefined criteria (Supplemental Table 1). Another investigator (C.-T. F.) independently verified the data coding and reviewed medical records to calculate the Carlson Comorbidities Index (CCI) [15].

Outcomes

In-hospital death was considered associated with NF if (i) it occurred before resolution of the signs and symptoms during patient admission, or (ii) death occurred within 30 days after the onset of NF without evidence of other causes of death, in accordance with global standardization of the definition for disease-related mortality in epidemiological surveillance [9,10,16].

Bacterial strains

NTUH routinely collected bacterial isolates from blood cultures and stored the strains at -80°C until use [8]. Based on the medical records of NF and the date of blood cultures, we retrospectively retrieved the stored bacterial strains that caused NF for molecular typing and virulence gene analysis. We analyzed the phylogenetic background of the strains that caused monomicrobial NF by performing multiplex polymerase chain reaction (PCR) as described previously [17]. Multilocus sequence typing was performed using the protocol based on that described by Wirth *et al.* [18].

Virulence gene profiles

E. coli strains that caused monomicrobial NF were evaluated for cytotoxic necrotizing factors 1 and 2 (*cnf1* and *cnf2*) as well as other extraintestinal pathogenic *E. coli* (ExPEC)-associated virulence genes, including toxins, adhesins, iron siderophores, and capsules [19]. *E. coli* isolates were categorized as ExPEC if they tested positive for one or more ExPEC-associated extraintestinal virulence factors [19]. ExPEC strains that harbored *cnf*1 or *cnf*2 were considered necrotoxigenic *E. coli* (NTEC) [20]. Sequences of the PCR primers, with literature references, are listed in Supplemental Table 2.

Statistical analysis

With an expected 1:1:1 ratio of patients with type I, type II, and type III NF, as well as an estimated NF-related mortality risk of 20% for type I and type II NF. A minimum of 228 patients would be required to detect a 20% increase in mortality risk in type III NF based on chi-square test with a power of 90%. Categorical data were compared using chi-square test or Fisher's exact test when the expected value of any cell in the 2×2 contingency table was less than five. Continuous data were compared using nonparametric Wilcoxon rank sum test. Survival curves were compared using log-rank test. We applied logistic regression to identify risk factors for NF-related mortality. For regression modeling, the maximum model included all variables in comorbidities, locations of NF,

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time to surgery, and causal microorganism(s), which had a *P*-value of <0.20 in the univariable analysis. We applied stepwise selection to choose the optimum model, with patient age and CCI forced into the final model. Statistical analyses were performed using SAS v9.4 (SAS Institute, Cary, NC, US). All analyses were two-sided, with $P < 0.05$ considered statistically significant.

Results

Baseline clinical data

A total of 235 consecutive patients with surgically confirmed NF were included in this study, with a median age of 61 years (interquartile range [IQR]: 50 to 72 years) and a male:female ratio of 154:81. Sixty-three patients (63/235, 26.8%) died from NF during the hospitalization (average follow-up time: 40.0 days; total follow-up time: 9406 patient-days), with a median time to death of 16 days (IQR: 3 to 28 days).

High mortality of type III necrotizing fasciitis

Type III NF (monomicrobial, gram-negative, proportion: 68/235, 28.9%) was associated with a two-fold higher mortality risk than that of type I NF (polymicrobial, proportion: 64/235, 27.2%), and type II NF (monomicrobial gram-positive, proportion: 79/235, 33.6%) (mortality risk: 42.6% vs 23.4% and 19.0%, $P = 0.019$ and 0.002, respectively, Table 1). Figure 1 shows the Kaplan-Meier estimates for the probability of death by NF types (type III vs type I: $P = 0.017$; type III vs type II: $P < 0.001$, log-rank test).

Mortality by causal microorganism

Table 1 shows the pathogen species-specific mortality risk. The highest case fatality rate was observed for *E. coli* (61.5%), followed by *K. pneumoniae* (40.0%), *A. hydrophila* (37.5%), and *V. vulnificus* (25.0%) (the four most common gram-negative bacteria of monomicrobial NF), in contrast to the case fatality rates observed in polymicrobial (23.4%), GAS (16.7%), and *S. aureus* (16.2%; in decreasing rank, $P = 0.028$, chi-square test; $P < 0.001$, chi-square test for trend).

Among the gram-negative bacteria that cause monomicrobial NF, *E. coli* was associated with a particularly high mortality risk (adjusted odds ratio [OR] 6.51, 95% confidence interval: 1.95 to 23.76, $P = 0.003$) after adjusting for the effects of patient age and comorbidities (including chronic kidney disease, cirrhosis, gout, and CCI; Table 2). Monomicrobial *K. pneumoniae* NF was associated with an increase in NF-related mortality (adjusted OR 2.31, $P = 0.104$), although statistically non-significant (Table 2).

Pathogen factors associated with poor outcome

The 3.7-fold higher case fatality rate of monomicrobial *E. coli* NF than that of GAS NF (61.5% vs 16.7%, $P = 0.01$) cannot be explained by the difference in age (median: 57 years vs 59.5 years), locations of NF, or comorbidities (chronic kidney diseases: 7.7% vs 20.8%; gout: 7.7% vs 8.3%; the number of immunocompromising conditions [i.e., diabetes mellitus, liver cirrhosis, nephrotic syndrome, alcoholism, or use of immunosuppressants]: median: 1 vs 1) (all $P > 0.05$; Table 3). Time to surgery was not different between the two groups ($P = 1.00$). In contrast, monomicrobial *E. coli* NF was more likely to have bacteremia at initial presentation than GAS NF (76.9% vs 33.3%, $P = 0.017$) and was more likely to present with sepsis-related neutropenia (23.1% vs 0%, $P = 0.037$; Table 3).

Some of the *E. coli* strains that caused monomicrobial NF were non-susceptible to third-generation cephalosporins (38.5%), piperacillin/tazobactam (30.8%), and fourth-generation cephalosporins (7.7%), respectively, although all the *E. coli* NF strains remained susceptible to carbapenems (Table 4). However,

Table 1
Mortality of necrotizing fasciitis, by causal microorganism(s).

Type of NF	Causal microorganism(s)	No. (% of total cases)	Death % (deaths in a category)
Type I (Polymicrobial)	See footnote[a]	64 (27.2)	23.4% (15/64)[b]
Type II (Monomicrobial, gram-positive)		79 (33.6)	19.0% (15/79)[b]
	Staphylococcus aureus	37 (15.7)	16.2% (6/37)[d]
	MSSA	18	5.6% (1/18)
	MRSA	19	26.3% (5/19)
	Streptococcus pyogenes	24 (10.2)	16.7% (4/24)[d]
	Others[c]	18 (7.7)	27.8% (5/18)[e]
Type III (Monomicrobial, gram-negative)		68 (28.9)	42.6% (29/68)*
	Klebsiella pneumoniae	20 (8.5)	40.0% (8/20)[d]
	Escherichia coli	13 (5.5)	61.5% (8/13)[d]
	Vibrio vulnificus	12 (5.1)	25.0% (3/12)[d]
	Aeromonas hydrophila	8 (3.4)	37.5% (3/8)[d]
	Others[f]	15 (6.4)	46.7% (7/15)[g]
Culture-negative		24 (10.2)	16.7% (4/24)
Total		235 (100.0)	26.8% (63/235)

Abbreviation: NF, necrotizing fasciitis; MSSA, methicillin-susceptible *Staphylococcus aureus*; MRSA, methicillin-resistant *S. aureus*.

[a] Mixed aerobic/anaerobic ($n = 43$), miscellaneous ($n = 21$). *E. coli* was involved in 19 of the 64 polymicrobial NF cases. The case fatality rate of polymicrobial *E. coli* NF was 5/19 (26.3%), not different from that of polymicrobial NF not involving *E. coli* (10/45, 22.2%) or polymicrobial NF in general (15/64, 23.4%) ($P = 0.753$ and 0.7691, respectively).

[b] Monomicrobial gram-negative NF had a mortality two-fold higher than polymicrobial or monomicrobial gram-positive NF (42.6% vs 23.4% or 19.0%, $P = 0.019$ and 0.002, respectively).

[c] Group B *Streptococcus* ($n = 3$), Group G *Streptococcus* ($n = 2$), anaerobic non-spore forming gram-positive bacilli ($n = 2$), *Enterococcus faecium* ($n = 2$), *Enterococcus species* ($n = 1$), *Staphylococcus haemolyticus* ($n = 1$), *Staphylococcus epidermidis* ($n = 1$), non-group ABD beta-streptococcus ($n = 1$), *Streptococcus anginosis* ($n = 1$), *Streptococcus constellatus* ($n = 1$), *Streptococcus species* ($n = 1$), *Slackia exigua* ($n = 1$), *Mycobacterium abscessus* (n = 1).

[d] Monomicrobial gram-negative NF had a mortality two-fold higher than polymicrobial or monomicrobial gram-positive NF (42.6% vs 23.4% or 19.0%, $P = 0.019$ and 0.002, respectively). Mortality differed by causal microorganism ($P = 0.028$ *Escherichia coli* [61.5%], *Klebsiella pneumoniae* [40.0%], *Aeromonas hydrophila* [37.5%], *Vibrio vulnificus* [25.0%], polymicrobial [23.4%], *Streptococcus pyogenes* [16.7%], *Staphylococcus aureus* [16.2%], $P < 0.001$ for trend).

[e] Two deaths from *Enterococcus faecium*, one death from group B *Streptococcus*, group G *Streptococcus*, and *Streptococcus species*, respectively.

[f] *Pseudomonas aeruginosa* ($n = 3$), *Serratia marcescens* ($n = 3$), *Proteus vulgaris* ($n = 2$), *Enterobacter cloacae* ($n = 2$), *Salmonella* O9 ($n = 1$), *Acinetobacter baumannii* ($n = 1$), *Pseudomonas fluorescens* ($n = 1$), non-O1 *Vibrio cholera* ($n = 1$), *Stenotrophomonas maltophilia* ($n = 1$).

[g] Two deaths from *Serratia marcescens*, one death from *Proteus vulgaris, Acinetobacter baumannii, Pseudomonas fluorescens*, non-O1 *Vibrio cholera*, and *Stenotrophomonas maltophilia*, respectively.

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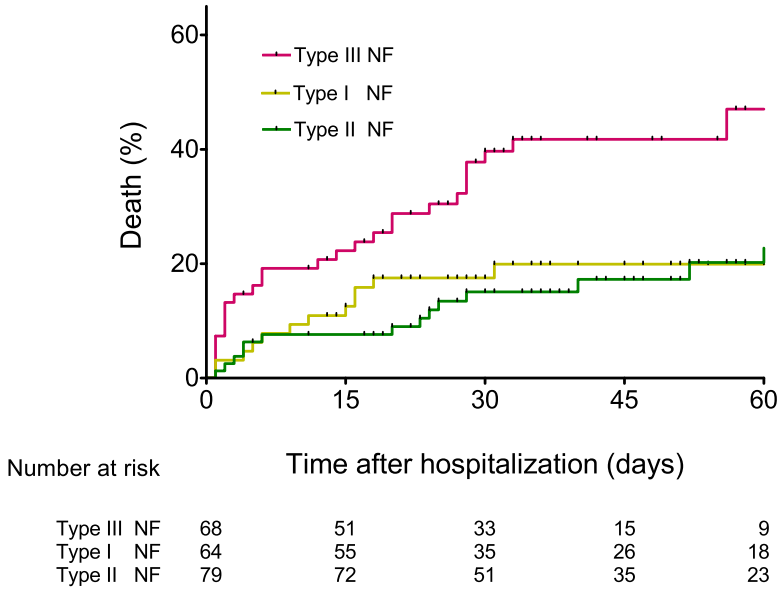

Figure 1. Kaplan-Meier estimates for the probability of death, by type I, type II, and type III NF. NF, necrotizing fasciitis.

the case fatality rate did not statistically differ between those who received initial appropriate antibiotic therapy and those who did not (5/9 [55.6%] vs 3/4 [75.0%], $P = 1.000$; Supplemental Table 3 and Supplemental Table 4). In contrast, all 24 GAS NF strains (100%) were susceptible to penicillin and were treated using initial appropriate antibiotic therapy.

Molecular profiles of E. coli in type III necrotizing fasciitis

E. coli strains from 8 of 13 monomicrobial *E. coli* NF cases were available for molecular analysis (strains from NF cases before 2006 were not available). The results showed no evidence of clonal spread (phylogenetic groups: B2, D, B2, B1, D, B2, D, and B2; sequence type: ST493, ST1605, ST567, ST3678, ST38, ST193, ST215, and ST131; Supplemental Table 5). All monomicrobial *E. coli* NF isolates are ExPEC harboring extraintestinal virulence genes, including one NTEC strain that produced *cnf1* and *cnf2* (Table 5).

Discussion

Our data, based on 235 consecutive patients with surgically confirmed NF, showed that type III NF had a two-fold higher mortality risk than that of type I and type II NF (42.6% vs 23.4% and 19.0%, $P = 0.019$ and 0.002, respectively). Our study shows for the first time that monomicrobial *E. coli* NF and monomicrobial *K. pneumoniae* NF had the highest mortality risk among type III NF (61.5% and 40.0%, respectively) and that monomicrobial *E. coli* NF is a significant predictor for NF-related mortality (adjusted OR 6.51, $P = 0.003$) even after adjusting for the effects of patient age and comorbidities. Our results strongly supports the inclusion of monomicrobial *E. coli* NF and monomicrobial *K. pneumoniae* NF into type III NF, as proposed by Kuehl *et al.* in 2019 [13].

The expansion of type III NF to include all monomicrobial NF caused by gram-negative bacteria will simplify the diagnostic algorithm for evaluating and managing NF. Clinicians can use a simple, inexpensive gram stain of wound samples to rapidly diagnose type III NF, which exhibits a high mortality risk. The rapid diagnosis of type III NF will inform empirical choices of antimicrobial agents, including a carbapenem to cover *E. coli* strains that are non-susceptible to third or fourth-generation cephalosporins or piperacillin-tazobactam. In contrast, the current definition of type III NF (*Vibrio* species or *Aeromonas* species) requires waiting for culture results and may miss the most life-threatening type of monomicrobial NF caused by *E. coli* or *K. pneumoniae*.

Our study provides new data on antimicrobial susceptibility and virulence gene profiles for type III NF caused by *E. coli*. This highlights that type III NF should not be understood solely as NF cases occurring in elderly patients with immunocompromising comorbidities. Instead, virulent gram-negative pathogenic bacteria with reduced susceptibility to antibiotics are behind the high mortality risk associated with this type of NF.

We found that all the monomicrobial *E. coli* NF isolates are ExPEC. Unlike commensal *E. coli* strains present in the gastrointestinal tract, ExPEC harbor virulence factors capable of causing extraintestinal diseases [19,20]. Extraintestinal virulence involves a diverse range of virulence factors, including adhesins, iron siderophores, capsules, and toxins [19]. The inclusive designation of "ExPEC" has replaced the older designations such as "uropathogenic *E. coli*" [19], because none of these virulence genes are specifically associated with the urinary tract or other particular sites of infection [21].

Extraintestinal virulence factors allow the hematogenous spread of ExPEC strains, as demonstrated by the high proportion of monomicrobial *E. coli* NF cases with bacteremia at the initial presentation (*E. coli* NF vs GAS NF: 76.9% vs 33.3%, $P = 0.017$). Bacteremia indicates a systematic spread of pathogens beyond local NF sites. This triggers profound severe sepsis, as indicated by the occurrence of severe sepsis-related neutropenia in *E. coli* NF and not in GAS NF (23.1% vs 0.0%, $P = 0.037$) (Table 3), consistent with the critical role of bacteremia in NF-related mortality [22].

NTEC, which produces *cnf1* or *cnf2*, is a highly virulent type of ExPEC [23]. *Cnf1* and *cnf2* cause necrosis in rabbit models and contribute to tissue invasion [24]. In addition to *cnf1* and *cnf2*, NTEC harbors various additional virulence genes (e.g., hemolysin, iron acquisition, adhesins, and serum resistance) [20]. Four (80%) of five reported *cnf1*-positive *E. coli* NF cases have been found to be fatal [23,25–27]. In our study, NF caused by an NTEC strain resulted in rapid death (case 5, Table 5). The most alarming observation is that NTEC strains are capable of causing fulminant and fatal NF outcomes in individuals without immunocompromising conditions, as observed in the case reported by Bekal *et al.* [23] and case 5 in the present study.

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Table 2
Risk factors for death from necrotizing fasciitis.

Variables	Survivors (n = 172)	Non-Survivors (n = 63)	Univariable analysis		Multivariable analysis	
			OR (95% CI)	*P*-value	Adjusted OR (95% CI)[b]	*P*-value
Age in years, median (IQR)	60 (20)	62 (19)	1.01 (0.99 to 1.03)	0.167	1.00 (0.97 to 1.02)	0.799
Sex						
Female	61 (35.5)	20 (31.7)	1.00 (Reference)		-	-
Male	111 (64.5)	43 (68.3)	1.18 (0.64 to 2.22)	0.595	-	-
Comorbidities						
Diabetes mellitus	97 (56.4)	35 (55.6)	0.97 (0.54 to 1.74)	0.909		
Chronic kidney disease	25 (14.5)	19 (30.2)	2.54 (1.27 to 5.03)	0.008[a]	2.39 (1.07 to 5.31)	0.032[a]
Cirrhosis	14 (8.1)	11 (17.5)	2.39 (1.00 to 5.58)	0.045[a]	2.18 (0.83 to 5.63)	0.107
Use of immunosuppressant	13 (7.6)	9 (14.3)	2.04 (0.80 to 5.00)	0.123	-	-
Hematologic disease	11 (6.4)	5 (7.9)	1.26 (0.38 to 3.63)	0.678		
Gout	10 (5.8)	9 (14.3)	2.70 (1.02 to 7.05)	0.041[a]	3.72 (1.32 to 10.47)	0.012[a]
Malignancy	12 (7.0)	4 (6.3)	0.90 (0.25 to 2.71)	0.866	-	-
Intravenous drug abuse	4 (2.3)	0 (0.0)	-	0.984	-	-
Charlson comorbidity index, median (IQR)	3 (3)	4 (3)	1.17 (1.03 to 1.33)	0.017[a]	1.10 (0.90 to 1.34)	0.335
Preceding local factors at NF sites	70 (40.7)	25 (39.7)	0.96 (0.53 to 1.72)	0.888	-	-
Locations of NF						
Lower limbs	119 (69.2)	41 (65.1)	1.00 (Reference)		-	-
Neck and trunk	21 (12.2)	13 (20.6)	1.80 (0.81 to 3.88)	0.140	2.86 (1.23 to 6.60)	0.014[a]
Upper limbs	16 (9.3)	5 (7.9)	0.91 (0.28 to 2.48)	0.857	-	-
Perineum	16 (9.3)	4 (6.3)	0.73 (0.20 to 2.11)	0.585	-	-
Symptoms/signs at presentation						
Pain	153 (89.0)	55 (87.3)	0.85 (0.36 to 2.17)	0.725	-	-
Swelling	151 (87.8)	56 (88.9)	1.11 (0.47 to 2.95)	0.818	-	-
Erythema	143 (83.1)	45 (71.4)	0.51 (0.26 to 1.01)	0.049[a]	-	-
Hypotension	47 (27.3)	31 (49.2)	2.58 (1.42 to 4.70)	0.002[a]	-	-
Dyspnea	51 (29.7)	27 (42.9)	1.78 (0.98 to 3.23)	0.058	-	-
Bullae	65 (37.8)	25 (39.7)	1.08 (0.60 to 1.95)	0.792	-	-
Open wound	67 (39.0)	25 (39.7)	1.03 (0.57 to 1.85)	0.919	-	-
Pus discharge	40 (23.3)	9 (14.3)	0.55 (0.24 to 1.17)	0.138	-	-
Crepitus	10 (5.8)	9 (14.3)	2.70 (1.02 to 7.05)	0.041[a]	-	-
Laboratory data at presentation						
Bacteremia	54 (31.4)	30 (47.6)	1.99 (1.10 to 3.59)	0.023[a]	-	-
Presence of acute kidney injury	88 (51.2)	49 (77.8)	3.34 (1.76 to 6.70)	<0.001[a]	-	-
Neutropenia (<1,500 μl)	3 (1.7)	5 (7.9)	4.86 (1.16 to 24.27)	0.034[a]	-	-
Creatinine (mg/dl), mean ± SD	1.9 ± 1.8	3.4 ± 5.4	1.23 (1.07 to 1.42)	0.004[a]	-	-
Hemoglobulin (g/dl), mean ± SD	11.6 ± 2.6	10.5 ± 2.7	0.85 (0.75 to 0.95)	0.005[a]	-	-
White blood cell (x10^3) (/ul), mean ± SD	16.1 ± 9.1	14.8 ± 10.7	1.00 (1.00 to 1.00)	0.373	-	-
CRP (mg/dl), mean ± SD[c]	20.8 ± 11.7	18.7 ± 10.4	0.98 (0.96 to 1.01)	0.257	-	-
Time to surgery						
<12 hours	81 (47.1)	27 (42.9)	1.00 (Reference)		-	-
>12 hours	91 (52.9)	36 (57.1)	1.19 (0.66 to 2.14)	0.564	-	-
First operation procedures						
With amputation	27 (15.7)	12 (19.0)	1.00 (Reference)		-	-
Without amputation	145 (84.3)	51 (81.0)	0.79 (0.38 to 1.73)	0.542	-	-
Causal microorganism(s)						
Polymicrobial	49 (28.5)	16 (25.4)	0.85 (0.43 to 1.62)	0.639	-	-
Escherichia coli	5 (2.9)	8 (12.7)	4.86 (1.56 to 16.66)	0.007[a]	6.51 (1.95 to 23.76)	0.003[a]
Streptococcus pyogenes	20 (11.6)	4 (6.3)	0.52 (0.15 to 1.43)	0.244	-	-
MRSA	14 (8.1)	5 (7.9)	0.97 (0.30 to 2.67)	0.960	-	-
MSSA	17 (9.9)	1 (1.6)	0.15 (0.01 to 0.74)	0.065	0.19 (0.01 to 1.11)	0.131
Klebsiella pneumoniae	12 (7.0)	8 (12.7)	1.94 (0.73 to 4.94)	0.170	2.31 (0.82 to 6.30)	0.104
Vibrio vulnificus	9 (5.2)	3 (4.8)	0.91 (0.20 to 3.15)	0.885	-	-
Aeromonas hydrophilia	5 (2.9)	3 (4.8)	1.67 (0.33 to 7.02)	0.491	-	-
Others[c]	41 (23.8)	15 (23.8)	1.00 (0.50 to 1.94)	0.996	-	-

Data are median (IQR), n (%), or mean ± SD.
Abbreviations: CI, confidence interval; CRP, C-reactive protein; IQR, interquartile range; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*; NF, necrotizing fasciitis; OR, odds ratio.
[a] Statistically significant.
[b] Adjusted using logistic regression.
[c] CRP data are missing in 37 survivors and nine non-survivors.[d]Other pathogens or culture-negative.

K. pneumoniae was not only the most common (20/68, 29.4%) causal microorganism of type III NF in the present study, but it also had the second highest case fatality rate (40.0%). Monomicrobial *K. pneumoniae* NF is also associated with a high risk of bacteremia, as well as an additional hazard of concurrent distant abscesses (via hematogenous spread) that may affect vital organs such as the central nervous system or the eyes [8,28]. Physicians who treat patients with monomicrobial *K. pneumoniae* NF should be aware of the high mortality rate and the potential catastrophic disabilities resulting from a septic eye or central nervous system complications. The reclassification of type III NF to include monomicrobial *K. pneumoniae* NF will greatly facilitate early recognition and timely management through a wound gram stain-based rapid diagnosis to differentiate high-risk, difficult-to-treat type III NF from the less dangerous type I or type II NF, instead of waiting for culture results.

The strength of the present study includes the large sample size powered to examine the prognostic value of expanded type III NF, comprehensive clinical and microbiological data, the independent verification of causal microorganism(s), as well as the determina-

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Table 3
Comparison of monomicrobial necrotizing fasciitis between patients infected with *Escherichia coli* and group A *Streptococcus*.

	E. coli (n = 13)	GAS (n = 24)	*P*-value
Age in years, median (IQR)	57.0 (18.0)	59.5 (34.5)	0.826
Male:female ratio	8:5	17:7	0.716
Comorbidities			
Diabetes mellitus	6 (46.2)	11 (45.8)	1.000
Chronic kidney disease	1 (7.7)	5 (20.8)	0.395
Cirrhosis	3 (23.1)	1 (4.2)	0.115
Use of immunosuppressant	1 (7.7)	2 (8.3)	1.000
Hematologic disease	0 (0.0)	0 (0.0)	-
Gout	1 (7.7)	2 (8.3)	1.000
Malignancy	0 (0.0)	1 (4.2)	1.000
Intravenous drug abuse	0 (0.0)	0 (0.0)	-
CCI [b], median (IQR)	4 (2)	2 (4)	0.171
Immunocompromising conditions [c]			
Any	9 [d] (69.2)	13 [e] (54.2)	0.491
Number, median (IQR)	1 (1)	1 (1)	0.326
Preceding local factors	3 [f] (23.1)	16 [g] (66.7)	0.017 [a]
Sites of NF			0.855
Lower limbs	12 (92.3)	20 (83.3)	
Upper limbs	0 (0.0)	2 (8.3)	
Neck or trunk	0 (0.0)	1 (4.2)	
Perineum	1 (7.7)	1 (4.2)	
Severity of sepsis syndrome [h]			
Sepsis-related hypotension	10 (76.9)	16 (66.7)	0.711
Sepsis-related organ dysfunction	8 (61.5)	10 (41.7)	0.313
Laboratory data			
Bacteremia	10 (76.9)	8 (33.3)	0.017 [a]
White blood cell (x 10^3) (/ul) ±SD	8.0 (6.8)	16.0 (11.2)	0.034 [a]
Neutropenia (<1,500 μl)	3 [i] (23.1)	0 (0)	0.037 [a]
Presence of AKI [j]	8 (61.5)	20 (83.3)	0.229
Creatinine (mg/dl) ±SD	1.9 (1.4)	2.4 (1.8)	0.293
Hemoglobulin (g/dl) ±SD	11.2 (3.0)	11.6 (2.8)	0.497
C-reactive protein (mg/dl) ±SD	16.3 (8.3)	22.2 (8.5)	0.080
Time to surgery			
< 12 hours	7 (53.8)	14 (58.3)	1.00
> 12 hours	6 (46.2)	10 (41.7)	
Outcomes			
Limb loss	3 (23.1)	8 (33.3)	0.711
Infection-related mortality [k]	8 (61.5)	4 (16.7)	0.010 [a]

Data are no. (%) of patients, unless otherwise indicated.
Abbreviations: AKI, acute kidney injury; CCI, Carlson Comorbidity Index; GAS, group A *Streptococcus*; NF, necrotizing fasciitis; IQR, interquartile range.

[a] P <0.05, chi-square test, Fisher's exact test, or Wilcoxon rank sum test.

[b] CCI (Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 1987; 40: 373-83.)

[c] Some patients had more than one condition.

[d] Diabetes mellitus (n = 6); liver cirrhosis (n = 2); alcoholism (n = 1); systemic lupus erythematosus (SLE), end-stage renal disease s/p renal transplantation, corticosteroids, cyclophosphamide pulse therapy, and rituximab for SLE flare-up (n = 1), alcoholic cirrhosis s/p liver transplantation (n = 1).

[e] Diabetes mellitus (n = 11); steroid abuse due to bilateral knee osteoarthritis (n = 1); periodic local steroid injection for bilateral knee osteoarthritis (n = 1); nephrotic syndrome (n = 1); liver cirrhosis (n = 1), lung adenocarcinoma s/p chemotherapy with cis-platin and docetaxel (n = 1).

[f] Saphenous vein harvest wound (n = 1); fall with leg laceration wound (n = 1); local analgesic injection and acupuncture (n = 1).

[g] Local injection (n = 1); traffic accident with left femur fracture (n = 1); ankle sprain (n = 1); foot ulcer (n = 1); leg ulcer (n = 1); cutting wound (n = 1); fall (n = 1); leg injury (n = 1); burn injury (n = 1); intra-uterine device infection with right salpingitis and acute suppurative infection of peri-tubal soft tissue s/p salpingectomy, complicated by post-operative NF over right inguinal area and right flank (n = 1); chronic leg wound (n = 1); fall with sprain (n = 1); scratching wound (n = 1); right foot chronic wound (n = 1); foot wound, bilateral (n = 1); leg wound after acupuncture (n = 1).

[h] Sepsis, sepsis-related hypotension, and organ dysfunction were defined based on the 1992 American College of Chest Physicians/Society of Critical Care Medicine Consensus (Critical Care Med 1992; 20: 864-874).

[i] Severe sepsis-related. None of the patients (cases 2, 9, and 10, see Supplemental Table 3) had underlying hematologic diseases or malignancy, or ever received chemotherapy before the occurrence of NF.

[j] AKI, acute kidney injury: an increase in serum creatinine by >0.3 mg/dl within 48 hours, an increase in serum creatinine to >1.5 times of baseline within 7 days, or urine volume < 0.5 ml/kg/h for 6 hours (Khwaja A. KDIGO clinical practice guidelines for acute kidney injury. Nephron Clin Pract 2012; 120: c179-84).

[k] Death was considered related to NF if (1) it occurred before resolution of the signs and symptoms, or (2) within 14 days of the onset of NF, while there being no evidence of other causes of death.

tion of bacterial virulence genes, and antimicrobial susceptibility. The number (and proportion) of type III NF cases in our study (68/235, 28.9%) are much higher than that in the prior study in Switzerland (9/60, 15.0%) [13] or South Korea (31/115, 27.0%) [9], which allows more detailed analysis at the level of individual bacterial species.

Our study has the following limitations. First, type III NF is a geographic phenomenon predominantly encountered in Asian countries, rather than observed universally worldwide. For example, no cases of type III NF were found in a recent study in the Netherlands [29]. The recommendation for adding type III in the classification of NF is arguably not universal.

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Table 4
Disk susceptibility of 13 *Escherichia coli* strains that caused monomicrobial necrotizing fasciitis.

Antimicrobial agents	Number (%) susceptible
Amoxicillin/clavulanate	5 (38.5)
Cefazolin	8 (61.5)
Cefmetazole	8 (61.5)
Cefotaxime	8 (61.5)
Cefepime	12 (92.3)
Piperacillin/tazobactam	9 (69.2)
Carbapenem [a]	13 (100.0)
Gentamicin	9 (69.2)
Amikacin	13 (100.0)
Fluoroquinolone[b]	9 (69.2)

[a] Imipenem or ertapenem.
[b] Ciprofloxacin or levofloxacin.

Second, this is a registry-based retrospective study of surgically confirmed NF. Nevertheless, surgeons at the Division of Plastic Surgery performed all surgical debridement for NF diagnosed and treated at NTUH, and the NF registry includes all surgically confirmed cases of NF. We did not have information on the rate of surgical confirmation among NF cases. In the South Korea monomicrobial NF study (n = 115), 68 (80%) of the 85 culture-positive NF cases were surgically confirmed [9]. NTUH has a comprehensive electronic database from which we can retrospectively obtain all clinical and laboratory data on NF patients during the study period, with the exception of C-reactive protein data, which is not a routine laboratory check-up and were missing in 37 survivors and nine non-survivors (Table 2, footnote). Third, this study was conducted at a single university medical center, in contrast to a national hospital database study in England which included 11,042 NF cases during 2002-2017 [30]. However, the nationwide database did not provide data on monomicrobial NF versus polymicrobial NF, bacterial virulence gene profile, and antimicrobial susceptibility, which are precisely the question that the present study aimed to answer. The patient characteristics in our study were similar to those in monomicrobial NF studies conducted in Israel [10] and South Korea [9]. In our study, the mean age was 60.9 years for type III NF patients and 58.5 years for type II NF patients. Among all NF patients, 56.2% had diabetes mellitus, 9.4% had immunosuppression, and 10.6% had liver cirrhosis. In the study conducted in Israel [10], the mean age was 59.7 years for type III NF patients and 58.0 years for type II NF patients. Among all NF patients, 75.6% had either diabetes mellitus or immunosuppression [10]. In the study conducted in South Korea [9], the median age was 59.0 years for type III NF patients and 53.0 years for type II NF patients. Among all NF patients, 45.9% had diabetes mellitus, 3.5% had immunosuppression, and 24.7% had liver cirrhosis [9]. The outcomes of type III NF (30-day mortality: 42.6% [29/68]) in our study were similar to those of studies conducted in Israel (30-day mortality: 42.1% [8/19]) [10] and South Korea (30-day mortality: 48.4% [15/31]) [9]. Therefore, despite being a single-center study, our findings on the prognostic value of type III NF may have good external validity.

Conclusion

Our findings support the expansion of current type III NF (NF caused by *Vibrio* spp. or *Aeromonas* spp.) to include all the monomicrobial NF caused by gram-negative bacteria, particularly *E. coli* and *K. pneumoniae*, proposed by Kuehl *et al.* in 2019 [13]. This not only provides a simplified framework that allows the use of simple gram stain to rapidly classify the NF types with prognostic

Table 5
Extraintestinal virulence genes of *Escherichia coli* strains isolated from patients with monomicrobial necrotizing fasciitis.

Immunocompromising conditions (Outcome)	Case 5 None (Died)	Case 6 None (Died)	Case 7 DM (Died)	Case 8 SLE (Survived)	Case 10 None (Died)	Case 11 DM (Survived)	Case 12 DM (Died)	Case 13 Liver Tx (Survived)
ST type	ST493	ST1605	ST567	ST3678	ST38	ST1193	ST215	ST131
Toxin								
cnf1	+	−	−	−	−	−	−	−
cnf2	+	−	−	−	−	−	−	−
hlyA	+	−	−	−	−	−	−	−
cdtB	−	−	+	−	−	−	−	−
Iron-capture								
iroN	+	−	+	+	−	−	+	−
iutA	−	−	−	−	−	+	−	+
fyuA	+	−	−	−	−	+	−	+
Capsular antigen								
K1	−	−	−	−	−	+	−	−
traT	+	+	−	+	−	−	+	+
kpsMTII	−	−	−	−	−	+	−	+
Protease								
ompT	+	+	−	−	−	+	−	+
Adhesins								
fimH	+	+	+	+	+	+	+	+
focG	+	−	−	−	−	−	+	−
papA	+	−	−	−	−	−	−	−
papG II	−	−	−	−	−	−	−	−
papG III	+	−	−	−	−	−	−	+
sfa/foc	+	−	−	−	−	−	−	−
afa/dra	−	−	−	−	−	−	−	−
hra	−	−	−	−	−	−	−	−
Others								
usp	+	−	+	−	−	+	−	+
tsh	−	+	−	−	−	+	−	−
malX	+	−	+	−	−	+	−	+

DM, diabetes mellitus; SLE, systemic lupus erythematosus, immunosuppressive therapy; ST, sequence type; Tx, transplantation; *cnf1* and *cnf2* (cytotoxic necrotizing factor 1 and 2), *cdtB* (cytolethal distending toxin), *hlyA* (hemolysin), *papA, papGII, papGIII, sfa/foc, fimH, focG, afa/dra* (adhesins/fimbriae), *iucA* (aerobactin), *iroN* (salmochelin), *fyuA* (yersiniabactin), K1 (group I capsule), *kpsMT* (group II capsule), *TraT* (resistance to serum), *ompT* (outer membrane protease), *hra* (heat-resistant agglutinin), *usp* (uropathogenic specific protein), *tsh*, and *malX* (pathogenicity island marker).

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implications but also informs the empirical antimicrobial choice to include a carbapenem in type III NF cases to cover multi-resistant *E. coli* strains. Moreover, our data further highlights that the high mortality risk of type III NF cannot be explained by comorbidities. Instead, type III NF represents an under-recognized threat from virulent gram-negative bacteria with reduced susceptibility to antimicrobials.

Declaration of competing interest

The authors have no competing interest to declare.

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Author contributions

NCC and CTF developed the study concept and study design. NCC, YC, HCT, and CTF collected clinical data. PRH provided the stored bacterial strains and performed molecular typing and virulence genes analyses. KLC, SHW, YHC, and CTF performed statistical analyses. NCC and CTF were the major contributors to writing the manuscript. All authors approved the final manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2023.04.390.

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