Emergence of Carbapenem-Resistant *Klebsiella pneumoniae* as a Cause of Necrotizing Skin and Soft Tissue Infections and Characterization of Associated Virulence Factors

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Background

- *Klebsiella pneumoniae* (KP) necrotizing skin and soft tissue infection (NSSTI) is an emergent disease[1,2,3] that has been associated with hypervirulent strains (hvKP) and the hypermucoviscosity phenotype (hmvKP)[4,5].
- Typically, carbapenem-resistant KP (CR-KP) strains are of low virulence and not associated with invasive infections such as NSSTI. However, few cases of NSSTI caused by CR-KP can be found in the recent literature[6], raising the concern for the emergence of KP clones that have managed to combine multidrug-resistance with high virulence traits.
- Clinical microbiology laboratories do not routinely screen for hmvKP or hvKP phenotypes, therefore identification of CR-KP strains with high virulence traits may go under-recognized.
- Consequently, virulence characterization of CR-KP NSSTI strains could unveil high virulence traits not detected in CR-KP to date.

Objectives

- Identify NSSTI caused by CR-KP among hospitalized patients of a US tertiary medical center and describe the demographic and clinical characteristics of patients with CR-KP NSSTI.
- Perform a comprehensive evaluation of the virulence of CR-KP NSSTI strains

Methods

- Retrospective study of all consecutive cases of CR-KP NSSTI diagnosed at a tertiary US hospital from Jan 2012 to Jan 2016.
- Demographic, clinical, and antibiotic susceptibility (Vitek) data, were obtained by chart review.
- Archived CR-KP isolates were tested for:
  - String test to evaluate for hypermucoviscosity.
  - Quantification of uronic acid production to measure of capsule production.
  - Whole genome sequencing (Illumina MiSeq) and *de novo* sequence assembling (SPAdes) for identification of antibiotic resistance genes (NCBI and ResFinder databases) and virulence genes* (Pasteur database).
  - *In silico* MLST (Resfinder) and capsular typing (using *wzc* sequencing).
  - *In vitro* virulence evaluation: serum killing assay and phagocytosis assay.
  - *In vivo* virulence evaluation: Pneumonia and subcutaneous abscess mouse models in immunocompetent and neutropenic mice (treated with Ly6G) using a low virulence (MGH-78578) and a hypervirulent (NTUH-K2044) control strains. Analysis of survival, abscess formation, bacterial burden at site of infection and dissemination to the liver was performed for each of the models. An inoculum of $5x10^6$ was used for all the models.

*Specific virulence genes evaluated: Hypermucoviscosity-associated genes (*rmpA*, *magA*); genes associated with iron acquisition systems (*iucC/iutA* or aerobactin, *ybt*, *entB*, *kfu*) and fimbrial genes (*mrkD*, *fimH*, *allS*).

Results

- A total of 4 cases of CR-KP NSSTI (average age 52 and average SOFA score 4) were identified during the study period.
- All patients were immunosuppressed or had diabetes mellitus.
- Polymicrobial NSSTI was identified in 2 out of 4 patients
- In-hospital mortality was 0%. However, 2 patients relapsed and 1 died due to CR-KP infection, yielding an infection-attributable mortality of 25%.

Table 1. Demographic, clinical and microbiologic characteristics of patients with CR-KP NSSTI

	NU-CRE101	NU-CRE176	NU-CRE212	NU-CRE265
Clinical Characteristics				
Age, Sex	69, M	50, F	46, M	43, M
Charlson Score	6	4	6	6
Specific Comorbidities	Bladder cancer on chemotherapy	Myasthenia Gravis on chronic corticosteroids	DM, CKD on dialysis, failed kidney and	DM, CKD on dialysis
Immunosuppresed	Yes	Yes	Yes	-
Surgical Infection	-	-	Yes	Yes
Modified SOFA score	2	4	7	4
Fever/Hypotension	Yes/No	No/Yes	Yes/Yes	No/No
Microbiologic characteristics				
Culture source	Tissue	Tissue/Blood	Tissue	Tissue
Polymicrobial culture	VRE	-	MSSA	-
Meropenem Susceptibility	R (>16)	R (>16)	R (>16)	R (>16)
Management				
Source control achieved (surgery)	yes	yes	yes	yes
Time to effective antibiotic (hs)	39.8	80.7	70.8	131.1
Outcomes				
Length of hospital stay (days)	23	6 (transferred)	13	55
In-hospital death	survived	survived	survived	survived
Relapse	-	after 4 months	after 5 months	-
Overall death (post-discharge)	**Yes**	-	**Yes**	-
Death attributable to infection	-	-	**Yes**	-

In vitro evaluation of virulence

- Only strain NU-CRE265 was found to be hmvKP by string test.
- Capsule production for the hmvKP strain NU-CRE265 was nearly twice that of other NSSTI strains and even higher than the hvKP control strain.

Figure 1. Strain NU-CRE265 positive string test

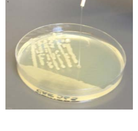
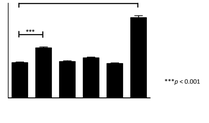


Figure 2. Capsule production measured by quantification of uronic acid.



- HmvKP NU-CRE265 was very sensitive to serum killing, but significantly resistant to phagocytosis, when compared to other strains, including controls.

Figure 3. Serum sensitivity. Bacterial CFU after 3 hours of exposure to pooled human serum.

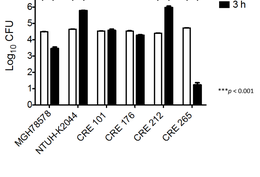
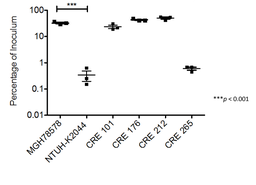


Figure 4. Phagocytosis assay. Bacterial uptake by macrophage-like J774 cells at 1 hour post infection.



In vivo evaluation of virulence

- NSSTI strains showed low virulence in the pneumonia mouse model, with 0% mortality, low bacterial burden and no dissemination (similar to the non-hvKP control) at day 4 post-infection.

Figure 5. Survival analysis in pneumonia mouse model

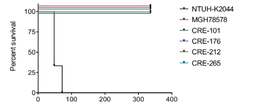
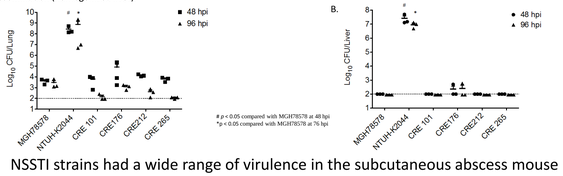


Figure 6. Bacterial burden at day 4 post-infection in the pneumonia mouse model. A, At site of infection (homogenized lungs). B, after dissemination (homogenized liver).



- NSSTI strains had a wide range of virulence in the subcutaneous abscess mouse model, with NU-CRE265 and NU-CRE176 forming larger abscesses with a bacterial burden similar to the hvKP control.
- Only NU-CRE265 showed dissemination to the liver similar to the hvKP control.
- In the neutropenic mice, a significant increase in abscess size, bacterial burden and dissemination was observed for all NSSTI strains and the non-hvKP control.

Figure 7. Area of abscess formation in the immunocompetent (IgG) and neutropenic (Ly6G) subcutaneous abscess mouse model

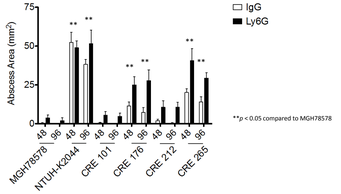
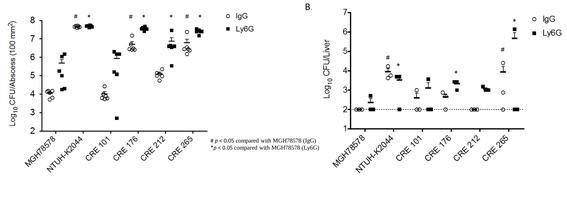


Figure 8. Bacterial burden at day 4 post-infection in the immunocompetent (IgG) and neutropenic (Ly6G) subcutaneous abscess mouse model. A, Bacterial burden within standardized abscess area. B, Bacterial burden in homogenized liver (dissemination)



Results

- All of the NSSTI strains had at least 2 virulence genes previously associated with hvKP strains (*mrkD* and *entB*), but all lacked the *rmpA* gene.
- The hmvKP NU-CRE265 strain belonged to the ST14 clone, K2 capsular type, and carried a KPC-3 gene in addition to other ESBL genes .

Table 2. Hypermucoviscosity and genotypic characterization of CR-KP NSSTI strains

	NU-CRE101	NU-CRE176	NU-CRE212	NU-CRE265
String test	-	-	-	**Pos**
MLST	ST-1082	ST-258	ST-258	ST-14
Capsule Serotype	K51	non-typable	non-typable	K2
Virulence genes				
rmpA	-	-	-	-
magA	-	-	-	-
mrkD	Pos	Pos	Pos	Pos
fimH	-	-	-	-
allS	-	-	-	-
entB	Pos	Pos	Pos	Pos
ybtS	-	-	Pos	-
kfuABC	Pos	-	-	Pos
iutA (Aerobactin receptor)	Pos	Pos	Pos	Pos
iucC (aerobactin synthetase)	-	-	-	-
Antibiotic resistance genes	KPC-2, SHV-1	KPC-3, SHV-182, TEM-1, OXA-9	KPC-3, SHV-182, TEM-1A	KPC-3, SHV-28, SHV-106, TEM-1, TEM-

Discussion

- CR-KP can cause invasive disease in the form of NSSTI, mostly in immunocompromised patients.
- The CR-KP NSSTI strains did not have the classical genotypic/phenotypic characteristics described in hvKP strains previously associated with NSSTI.
- However, different virulence traits were found among these CR-KP NSSTI strains, including presence of multiple virulence genes, increased capsule production, resistance to phagocytosis and potential for bacterial dissemination.
- Moreover, in the context of an abscess model, some CR-KP NSSTI isolates were as virulent as the hvKP control strain, suggesting that some of these isolates may be adapted to cause SSTIs.
- This study was limited by the small number of strains, however the in-depth analysis of virulence performed should provide a platform for further hypothesis-generation and studies in this area.
- This study adds to recent reports of CR-hvKP strains, by reporting the first carbapenem-resistant hmvKP causing NSSTI, as well as the first report of a hmvKP KPC-3 strain (NU-CRE265), and suggests that CR-hvKP strains with increased virulence may be emerging.

Conclusions

- CR-KP is an emergent cause of NSSTI in the US. These strains carry some virulence traits previously described in susceptible hvKP strains, although their role in the pathogenesis of NSSTI needs to be further studied.
- These results suggest that CR-KP strains with increased virulence may be emerging.

References

1. Cheng NC ,et al. Clin Infect Dis. 2012 Oct;55(7):930-9.
2. Bernal NP, et al. Burns 38 (2012) 252-256.
3. Lee SS. Clin Infect Dis. 2012; 55(7):940-2.
4. Mita N, et al. J Infect Chemother. 2012 Aug;18(4):565-8.
5. Ng D, et al. West J Emerg Med. 2015 Jan;16(1):165-8.
6. Rana MM, et al. Transpl Infect Dis 2013: 15: E157-E163.