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Antibiotic Resistance Coming Soon to A Pathogen Near You

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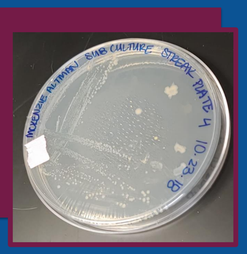

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McKenzie Altman, Tamia Mills, Lindsey Layman, Catherine Stone

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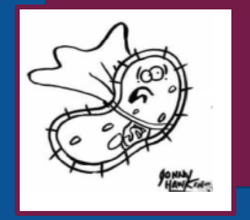


Abstract

The phenomena of antibiotic resistant bacteria has increased worldwide and can frequently be found on everyday surfaces. Here, we characterize a bacterial isolate from a woman's restroom door handle at Marian. Preliminary results comprised of morphological and biochemical tests, including growth characteristics on blood agar medium, as well as usage of the Kirby Bauer agar diffusion test to determine antibiotic susceptibility, identifies this isolate to be a multidrug resistant *Neisseria* species. However, additional detailed analysis is needed to confirm its identity.

Background of *Neisseria* spp.

Neisseria are commonly found in humans and animals as part of their natural microbiota. Many *Neisseria* spp. colonize the oral cavity and nasopharynx in humans. These are gram-negative diplococci which grow aerobically between 30-37°C. *Neisseria* are known fastidious organisms that grow well on blood and chocolate agar medium. Though both pathogenic and non-pathogenic or commensal strains of *Neisseria* are known, the distribution of pathogenic strains are of special concern. *Neisseria* bacteria is the causative agent of both meningitis and gonorrhoeae. *Neisseria meningitidis* is a leading cause of bacterial meningitis in children and young adults. Currently, six serogroups of this bacterium are responsible for most meningococcal disease worldwide. In addition, *N. gonorrhoeae* is one of the most frequently reported infectious diseases worldwide. The gonococcus adapts to the immune system of its host at an extraordinary rate, resulting in repeat infections. *N. gonorrhoeae* has the ability to develop resistance to all clinically used antibiotics thus it is an important pathogen in the study of antibiotic resistance. Rapid tests have been developed to identify and distinguish *N. gonorrhoeae* from the commensal species.

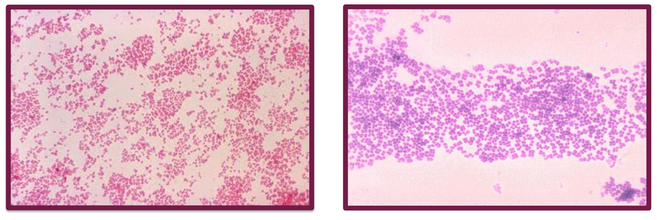


Figure 1. *Neisseria spp.* microscopy morphology examples.

Materials & Methods

Isolation
- Woman's bathroom door handle (WBD)
- Sterile cotton swab

Microscopy/Staining
- Gram Staining
- Capsule Staining
- Acid Fast Staining

Growth Media
- Blood Agar
- Mannitol Salt Agar (MSA)
- Eosin Methylene Blue Agar
- MacConkey's Medium

Biochemical Tests
- Oxidase
- Catalase
- IMViC
- Triple-Sugar Iron Agar (TSI)
- MRVP Medium
- Sulfide Indole Motility Medium (SIM)
- Antibiotic Sensitivity Test (AST)

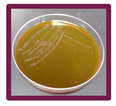


Figure 2. WBD EMB growth (left) and WBD morphological colony growth (right).

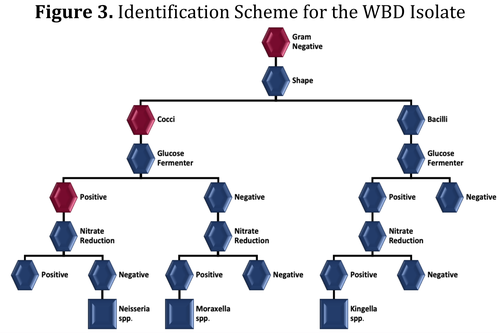
Biochemical Testing & Identification Results

Table 1. Morphological and biochemical characteristics of WBD isolate compared to *Neisseria and Moraxella spp.*

	WBD isolate	*Neisseria gonorrhoea*	*Moraxella catarrhalis*
Morphology	Small Diplococci	Small Diplococci	Large Diplococci
Gram Stain	Negative	Negative	Negative
Motility	Negative	Negative	Negative
Capsule	Positive	Negative*	Negative
Catalase	Positive	Positive	Positive
Oxidase	Positive	Positive	Positive
Hemolysis	Negative	Negative	Negative
Endospore	Negative	Negative	Negative
Acid-Fast	Negative	Negative	Negative
H2S	Negative	Negative	N/A
Gas Production	Negative	Negative	Negative
MSA	Negative	N/A	N/A
EMB	Positive	N/A	N/A
MacConkey	No growth	N/A	No growth
TSI	Negative	N/A	N/A
Citrate	Negative	N/A	Negative
SIM	Negative	N/A	Negative
MRVP	Negative	N/A	N/A
Fermentation of Sugars	Glucose only	Glucose only	None

*Scientific research indicates variable encapsulation results for *N. gonorrhoeae.*

Figure 3. Identification Scheme for the WBD Isolate



Kirby-Bauer Antimicrobial Test Results

Figure 4. Showing Mueller Hinton medium with antibiotic disks.

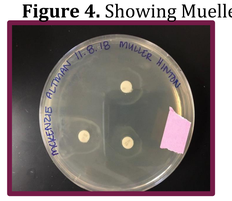


Table 2. Antibiotic Susceptibility of WBD isolate compared with *N. gonorrhoeae.*

Antibiotics (disc code)	WBD Isolate	*Neisseria gonorrhoeae*	Antibiotics (disc code)	WBD Isolate	*Neisseria gonorrhoeae*
Amikacin (AN30)	R	R	**Ampicillin (AM10)**	R	-
Cefoxitin (FOX30)	R	R	**Cephalothin (CF30)**	R	-
Ceftriaxone (CRO30)	R	I	**Imipenem (IPM10)**	R	S
Chloramphenicol (C30)	R	S	**Nalidixic Acid (NA30)**	R	S
Ciprofloxacin (CIP5)	R	R	**Novobiocin (NB30)**	I	-
Erythromycin (E15)	I	I	**Streptomycin (S10)**	R	I
Penicillin (P2)	R	R	**Sulfisoxazole (G.25)**	R	-

Conclusions

- WBD isolate showed poor growth on nutrient agar compared to blood agar.
- WBD isolate shares many morphological and biochemical characteristics with *Neisseria* spp.
- WBD isolate showed multidrug resistance and was even resistant to Imipenem, Nalidixic acid and Chloramphenicol in contrast to *N. gonorrhoeae.*

Literature Cited

Martens, E., & Demain, A. L. (2017). The antibiotic resistance crisis, with a focus on the United States. *The Journal of Antibiotics, 70*, 520–526. Retrieved from http://www.nature.com/ja

Trends in U.S. Antibiotic Use. (2017). Retrieved from Pew Charitable Trustswebsite:http://www.pewtrusts.org/en/research-and-analysis/issue-briefs/2017/03/trends-in-us-antibiotic-use

United States Antibiogram. (n.d.). Retrieved from https://wikem.org/wiki/United_States_Antibiogram