BIOGRAPHICAL SKETCH

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NAME: Robert A. Nicholas

eRA COMMONS USER NAME (credential, e.g., agency login): ROBERT_NICHOLAS

POSITION TITLE: Professor and Vice Chair

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Illinois, Urbana-Champaign	B.S. w/ high distinction	1977	Biochemistry
University of California, San Diego	Ph.D.	1984	Chemistry/Biochemistry
Harvard University, Cambridge, MA (Postdoctoral Training)	- - -	1984-1988	Biochemistry

A. Personal Statement

At UNC, I have built a research group that has defined at the molecular level the mechanisms of chromosomally mediated antibiotic resistance in *N. gonorrhoeae*. My laboratory utilizes a wide range of approaches, including genetics, molecular biology, bacteriology, structural biology, and biochemistry. We have established experimental systems that allow us to define the contributions of each determinant to antibiotic resistance, to identify the mutations in each determinant that confer resistance, and to quantify the synergism between these determinants in the acquisition of resistance. Our work in this area and with identifying important mutations in mosaic Penicillin-Binding Protein 2 (PBP2; the main target of β-lactam antibiotics in *N. gonorrhoeae*) has helped the field focus on surveillance and epidemiology to identify new outbreaks of antibiotic-resistant *N. gonorrhoeae*.

Much of my work has been done in collaboration. Our structural studies with Dr. Christopher Davies at the Univ of South Alabama have identified the molecular mechanisms by which mosaic PBP2 has remodeled its active site to discriminate against ceftriaxone. I also have active collaborations with Dr. William Shafer at Emory University on the function of the PG hydrolase, NlpC, in gonococcal growth and viability, with Drs. Ann Jerse at Uniformed Services University and Yonatan Grad at Harvard University on the role of compensatory mutations in the development of antibiotic resistance, and with Dr. Alex Duncan (a co-PI on this MPI proposal) at UNC investigating how the outer membrane porin, PorB, inhibits antigen presentation and dendritic cell-mediated T cell proliferation, as part of a long-range goal to understand how *N. gonorrhoeae* evades the human immune system and also how to make outer membrane vesicle vaccines more effective.

Ongoing projects:

1 R01 AI164794
Davies (PI), Role, co-investigator
04/01/2022-03/31/2027
Molecular mechanism of cephalosporin resistance in *N. gonorrhoeae* conferred by mutated PBP2

U19 AI144180-01
Jerse (PI), Role: Investigator
03/01/2019 – 02/28/2024
Nanodisc-displayed protein vaccines

1 R01 AI153521
Grad, Nicholas, Jerse (multi-PI)
07/01/2020 - 06/30/2025
Identification and analysis of compensatory mutations that support the evolution of antibiotic resistance in *Neisseria gonorrhoeae*

Citations:

a. Fenton BA, Tomberg J, Sciandra CA, Nicholas RA*, Davies C*, Zhou P*. (2021) Mutations in PBP2 from ceftriaxone-resistant Neisseria gonorrhoeae alter the dynamics of the β3-β4 loop to favor a low-affinity drug-binding state. J Biol Chem, **297(4):**101188. **PMC8503634**
b. Singh A, Turner JM, Tomberg J, Fedarovich A, Unemo M, Nicholas RA*, and Davies C*. (2020) Mutations in penicillin-binding protein 2 from cephalosporin-resistant *Neisseria gonorrhoeae* hinder ceftriaxone acylation by restricting protein dynamics. J Biol Chem, **295(21)**:7529-7543 **PMC7247294**
c. Zhu W, Tomberg J, Knilans KJ, Anderson JE, McKinnon KP, Sempowski GD, Nicholas RA*, Duncan JA*. (2018) Properly folded and functional PorB from *Neisseria gonorrhoeae* inhibits dendritic cell stimulation of CD4+ T cell proliferation. J Biol Chem. **293(28)**:11218-11229. **PMC6052219**
d. Vincent LR, Kerr SR, Tan Y, Tomberg J, Raterman EL, Dunning Hotopp JC, Unemo M, Nicholas RA*, Jerse AE*. (2018). In Vivo-Selected Compensatory Mutations Restore the Fitness Cost of Mosaic *penA* Alleles That Confer Ceftriaxone Resistance in *Neisseria gonorrhoeae*. *mBio* **9**, e01905-01917. **PMC588503**

* Co-corresponding authors

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

1988-1994	Assistant Professor, Department of Pharmacology, University of North Carolina at Chapel Hill
1994-2002	Associate Professor, Department of Pharmacology, University of North Carolina at Chapel Hill
2002-present	Professor, Department of Pharmacology, University of North Carolina at Chapel Hill
2006-present	Vice-Chair, Department of Pharmacology, University of North Carolina at Chapel Hill
2008-present	Professor, Department of Microbiology & Immunology, University of North Carolina at Chapel Hill
2013-present	Associate Director of Graduate Education
2013-2018	Faculty Director, Biological and Biomedical Sciences Program,

Other Experience and Professional Memberships

1992-present	Member, American Society for Microbiology
1993-1996	American Heart Association Research Review Committee, North Carolina Affiliate
1996-1998	American Heart Association Research Committee, North Carolina Affiliate
1996-present	Member, American Society for Pharmacology and Experimental Therapeutics (ASPET)
1998-2000	Member, ASPET Program Committee
1999-2018	Editorial Board, Molecular Pharmacology
1999-2004	Director of Graduate Studies, Dept. of Pharmacology
2005-2021	Program Director, Pharmacological Sciences Training Program T32
2001-2003	Secretary-Treasurer of ASPET Division for Molecular Pharmacology (elect, current, and past)
2005-2007	President of ASPET Division for Molecular Pharmacology (elect, current, and past)
2005-2018	Editorial Board, American Journal of Physiology
2007-2010	Executive Committee, ASPET Division for Molecular Pharmacology
2012-present	Associate Director of Graduate Education, UNC School of Medicine
2017-present	Nominator for HHMI Gilliam Fellowship awards for UNC
2021-present	Faculty Director of Admissions, Biological and Biomedical Sciences Program

Honors

1989-1991	Faculty Development Award in Basic Pharmacology from the Pharmaceutical Manufacturers Association Foundation
1992	Jr. Faculty Development Award from the University of North Carolina at Chapel Hill
1994-1999	Established Investigator of the American Heart Association
2003-2004	Recipient, Teaching Excellence Award in Pharmacology
2004-2005	Recipient, Teaching Excellence Award in Pharmacology
2016	Recipient, Outstanding Teaching Award
2017	Recipient, AAMC Innovation in Research and Research Education Award

C. Contributions to Science

1. Working closely with my collaborator, Dr. Christopher Davies, I have focused much of my effort over the last 10 years to understand the structural mechanisms and implications of antibiotic resistance mediated by penicillin-binding protein 2 (PBP2). The overarching goal of this work is to understand how mutations remodel PBP2 to drastically lower its rates of acylation with ceftriaxone and cefixime (up to 10,000-fold) without decreasing the essential transpeptidase activity of the enzyme below the threshold required for cell viability. These changes must be specific and subtle, as β-lactam antibiotics are substrate analogs of the peptide substrate. We have identified the mutations responsible for conferring resistance and have put together a structural roadmap on how these resistance mutations work together to decrease acylation rates with β-lactam antibiotics.
    a. Singh A, Tomberg J, Nicholas RA, and Davies C. (2019) Recognition of the β-Lactam carboxylate triggers formation of the acylated state of *N. gonorrhoeae* Penicillin-Binding Protein 2. J Biol Chem, **294(38)**:14020-14032. **PMC6755799**
    b. Tomberg J, Fedarovich A, Vincent LR, Jerse AE, Unemo M, Davies C*, Nicholas RA* (2017) Alanine-501 Mutations in Penicillin-Binding Protein 2 from Neisseria gonorrhoeae: Structure, Mechanism, and Effects on Cephalosporin Resistance and Biological Fitness. Biochemistry 56(8):1140-1150. **PMC5502787**
    c. Fedarovich A, Cook E, Tomberg J, Nicholas RA*, Davies C*. (2014) Structural effect of the Asp345a insertion in penicillin-binding protein 2 from penicillin-resistant strains of *Neisseria gonorrhoeae*. Biochemistry 53(48):7596-603. **PMC4263433**
    d. Tomberg J, Unemo M, Ohnishi M, Davies C*, Nicholas RA* (2013) Identification of the amino acids conferring high-level resistance to expanded-spectrum cephalosporins in the *penA* gene from the *Neisseria gonorrhoeae* strain H041. Antimicrob Agents Chemother 57(7):3029-36. **PMC3697319**
    e. Tomberg J, Temple B, Fedarovich A, Davies C*, Nicholas RA* (2012) A highly conserved interaction involving the middle residue of the SXN active-site motif is crucial for function of class B penicillin-binding proteins: mutational and computational analysis of PBP 2 from *Neisseria gonorrhoeae.* Biochemistry 51(13):2775-84. **PMC3338128**
2. My early work in chromosomally mediated antibiotic resistance in the pathogenic organism *Neisseria gonorrhoeae* focused on identifying the molecular basis of resistance. My lab was the first to identify the mutations responsible for resistance to β-lactam antibiotics and tetracyclines conferred by *penB*, *ponA*, and *rpsJ*. Screening for these mutations in clinical isolates is now commonplace in clinical microbiology labs throughout the world. These studies also revealed the complex nature of high-level antibiotic resistance and the synergistic interactions of different resistance determinants. For example, we showed that resistance due to mutations in the outer membrane porin PIB encoded by *penB* require the presence of the *mtr* determinant that increases expression of the MtrC-MtrD-MtrE efflux pump (this work is particularly relevant to the current proposal), that a mutation in PBP1 depends on other factors to increase resistance, and that *rpsJ* requires the *mtr* and *penB* determinants to achieve donor levels of resistance to tetracycline.
    a. Ropp PA, Hu M, Olesky M, Nicholas RA. (2002) Mutations in *ponA*, the gene encoding penicillin-binding protein 1, and a novel locus, *penC*, are required for high-level chromosomally mediated penicillin resistance in *Neisseria gonorrhoeae*. Antimicrob Agents Chemother, 46(3):769-77. **PMC127492**.
    b. Olesky M, Hobbs M, Nicholas RA. Identification and analysis of amino acid mutations in porin IB that mediate intermediate-level resistance to penicillin and tetracycline in *Neisseria gonorrhoeae*. (2002)

Antimicrob Agents Chemother, 46(9):2811-20. **PMC127413**.

c. Olesky M, Zhao S, Rosenberg RL, Nicholas RA. (2006) Porin-mediated antibiotic resistance in *Neisseria gonorrhoeae*: ion, solute, and antibiotic permeation through PIB proteins with *penB* mutations. J Bacteriol 188(7):2300-8. **PMC1428387**.

d. Hu M, Nandi S, Davies C, Nicholas RA. (2005) High-level chromosomally mediated tetracycline resistance in *Neisseria gonorrhoeae* results from a point mutation in the *rpsJ* gene encoding ribosomal protein S10 in combination with the *mtrR* and *penB* resistance determinants. Antimicrob Agents Chemother, 49(10):4327-34. **PMC1251527**.

3. Our more recent work with chromosomally mediated antibiotic resistance in *N. gonorrhoeae* has focused on understanding the mechanisms of this resistance. These papers are widely cited and are the definitive studies in the field for understanding chromosomally mediated resistance to the expanded-spectrum cephalosporins, ceftriaxone and cefixime. The studies also include our discovery of the role of the secretin PilQ in mediating influx of antibiotics into the cell.

   a. Zhao S, Duncan M, Tomberg J, Davies C, Unemo M, Nicholas RA. (2009) Genetics of chromosomally mediated intermediate resistance to ceftriaxone and cefixime in *Neisseria gonorrhoeae*. Antimicrob Agents Chemother 53(9):3744-51. **PMC2737842**

   b. Zhao S, Tobiason D, Seifert HS, and Nicholas RA (2005) The *penC* mutation conferring antibiotic resistance in *Neisseria gonorrhoeae* arises from a mutation in the PilQ secretin that interferes with multimer assembly and prevents antibiotic influx. Mol Microbiol, 57:1238-51. **PMC2673695**

   c. Nandi S, Swanson S, Tomberg J, Nicholas RA. Diffusion of antibiotics through the PilQ secretin in *Neisseria gonorrhoeae* occurs through the immature, SDS-labile form (2015) J Bacteriol, Jan 20;PubMed PMID: 25605303. **PMC4372736**

4. In collaboration with Drs. Bill Gutheil and Christopher Davies, we investigated the mechanisms and activities of the carboxypeptidase (CPase) PBP5 from *E. coli* and the CPases/endopeptidases PBP3 and PBP4 from *N. gonorrhoeae*. In a series of papers, we examined the structure of PBP5 and its enzymatic mechanism for carboxypeptidase and β-lactamase activity. These studies were facilitated by the structure of PBP5 bound to a transition state analog and biochemical studies to describe the mechanism of this enzyme. We also probed the function of gonococcal PBPs 3 and 4, and determined the physiological consequences on *N. gonorrhoeae* lacking these enzymes.

   a. Nicola, G., Peddi, S., Stefanova, M., Nicholas, R.A., Gutheil, W.G., and Davies, C. (2005) Crystal structure of *Escherichia coli* PBP 5 bound to a tripeptide boronic acid inhibitor: a role for Ser110 in deacylation. Biochemistry, 44(23):8207-17.

   b. Nicholas, R.A., Krings, S., Tomberg, J., Nicola, G., and Davies, C. (2003) Crystal Structure of Wild-type Penicillin-Binding Protein 5 at 1.85 Å Resolution: Implications for Deacylation. J. Biol. Chem., 278:52826-33.

   c. Stefanova, M., Tomberg, J., Olesky, M., Höltje, J.V., Gutheil, W.G., and Nicholas, R.A. (2003) *Neisseria gonorrhoeae* Penicillin-Binding Protein 3 exhibits exceptionally high carboxypeptidase and beta-lactam binding activities. Biochemistry, 42:4614-25.

   d. Stefanova, M., Tomberg, J., Davies, C., Nicholas, R.A., and Gutheil, W.G. (2003) Overexpression and Enzymatic Characterization of *Neisseria gonorrhoeae* Penicillin-Binding Protein 4. Eur J Biochem, 271:23-32.

A complete bibliography can be found through my NCBI at the following URL:
http://www.ncbi.nlm.nih.gov/sites/myncbi/10ap9uOKouf/bibliography/40331898/public/?sort=date&direction=ascending